This report presents the recommendations of a WHO Expert Committee commissioned to coordinate activities leading to the adoption of international recommendations for the production and control of vaccines and other biological substances, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of general issues brought to the attention of the Committee. The next part of the report, of particular relevance to manufacturers and national regulatory authorities, outlines the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, a WHO guidance document on Regulatory assessment of approved rDNA-derived biotherapeutics was adopted along with WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions and on WHO good manufacturing practices for biological products. In addition, revised WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines were also adopted by the Committee.

Subsequent sections of the report provide information on the current status and proposed development of international reference materials in the areas of antibiotics; biotherapeutics other than blood products; blood products and related substances; in vitro diagnostic device reagents; and vaccines and related substances.

A series of annexes are then presented which include an updated list of all WHO Recommendations, Guidelines and other documents on biological substances used in medicine (Annex 1). The above four WHO documents adopted on the advice of the Committee are then published as part of this report (Annexes 2–5). Finally, all additions and discontinuations made during the 2015 meeting to the list of International Standards, Reference Reagents and Reference Panels for biological substances maintained by WHO are summarized in Annex 6. The updated full catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/.
The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO’s constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

The Organization seeks through its publications to support national health strategies and address the most pressing public health concerns of populations around the world. To respond to the needs of Member States at all levels of development, WHO publishes practical manuals, handbooks and training material for specific categories of health workers; internationally applicable guidelines and standards; reviews and analyses of health policies, programmes and research; and state-of-the-art consensus reports that offer technical advice and recommendations for decision-makers. These books are closely tied to the Organization’s priority activities, encompassing disease prevention and control, the development of equitable health systems based on primary health care, and health promotion for individuals and communities. Progress towards better health for all also demands the global dissemination and exchange of information that draws on the knowledge and experience of all WHO’s Member countries and the collaboration of world leaders in public health and the biomedical sciences.

To ensure the widest possible availability of authoritative information and guidance on health matters, WHO secures the broad international distribution of its publications and encourages their translation and adaptation. By helping to promote and protect health and prevent and control disease throughout the world, WHO’s books contribute to achieving the Organization’s principal objective – the attainment by all people of the highest possible level of health.

The WHO Technical Report Series makes available the findings of various international groups of experts that provide WHO with the latest scientific and technical advice on a broad range of medical and public health subjects. Members of such expert groups serve without remuneration in their personal capacities rather than as representatives of governments or other bodies; their views do not necessarily reflect the decisions or the stated policy of WHO.

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WHO Expert Committee on Biological Standardization

Sixty-sixth report

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.
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WHO Expert Committee on Biological Standardization
12 to 16 October 2015

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Abbreviations

AEFI  adverse event following immunization
Ag/Ab  antigen/antibody
ALIFAR  Asociación Latinoamericana de Industrias Farmacéuticas
ATMP  advanced therapy medicinal product
BCG  bacille Calmette–Guérin
BGTD  Biologics and Genetic Therapies Directorate
BQ  Biological Qualifier (scheme)
BRN  WHO Blood Regulators Network
BSP  EDQM biological standardization programme
CBER  Center for Biologics Evaluation and Research
CIN  cervical intraepithelial neoplasia
CIN2–3  cervical intraepithelial neoplasia grades 2 or 3
CIN2+  cervical intraepithelial neoplasia grade 2 or worse
cLIA  competitive Luminex immunoassay
CMI  cell-mediated immunity
CTC  controlled temperature chain
DCVMN  Developing Countries Vaccine Manufacturers Network
DCVRN  Developing Country Vaccine Regulators’ Network
DNA  deoxyribonucleic acid
ECTC  extended controlled temperature conditions
EDQM  European Directorate for the Quality of Medicines & HealthCare
EGA  European Generic Medicines Association
EIA  enzyme immunoassay
ELISA  enzyme-linked immunosorbent assay
EMA  European Medicines Agency
EMP  WHO Department of Essential Medicines and Health Products
EPO  erythropoietin
EU  ELISA Unit(s)
FIX    factor IX
GAPIII  WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use
GCP    good clinical practice
GCV    geometric coefficient of variation
GMC    geometric mean concentration
GMP    good manufacturing practice(s)
GMT    geometric mean titre
GP     glycoprotein
HAV    hepatitis B virus
HBsAg  hepatitis B surface antigen
HBV    hepatitis B virus
HCV    hepatitis C virus
HEPA   high-efficiency particulate air
HEV    hepatitis E virus
HIV    human immunodeficiency virus
holoTC holotranscobalamin
HPV    human papillomavirus
HVAC   heating, ventilation and air conditioning
IARC   WHO International Agency for Research on Cancer
ICDRA  International Conference of Drug Regulatory Authorities
ICP    immune correlate of protection
ICRS   International Chemical Reference Substances
IFPMA  International Federation of Pharmaceutical Manufacturers & Associations
IgA    immunoglobulin A
IgE    immunoglobulin E
IgG    immunoglobulin G
IgM    immunoglobulin M
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>IGPA</td>
<td>International Generic Pharmaceutical Alliance</td>
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<tr>
<td>IIIV</td>
<td>inactivated influenza vaccine</td>
</tr>
<tr>
<td>INN</td>
<td>international nonproprietary name(s)</td>
</tr>
<tr>
<td>IPV</td>
<td>inactivated poliomyelitis vaccine</td>
</tr>
<tr>
<td>ISA</td>
<td>international standard for antibiotics</td>
</tr>
<tr>
<td>ISTH</td>
<td>International Society on Thrombosis and Haemostasis</td>
</tr>
<tr>
<td>IU</td>
<td>International Unit(s)</td>
</tr>
<tr>
<td>IVD</td>
<td>in vitro diagnostic</td>
</tr>
<tr>
<td>LAL</td>
<td>limulus amebocyte lysate</td>
</tr>
<tr>
<td>LB</td>
<td>lower bound</td>
</tr>
<tr>
<td>Lf</td>
<td>limit of flocculation</td>
</tr>
<tr>
<td>LL</td>
<td>lower limit</td>
</tr>
<tr>
<td>LLOD</td>
<td>lower limit of detection</td>
</tr>
<tr>
<td>LLOQ</td>
<td>lower limit of quantification</td>
</tr>
<tr>
<td>LMIC</td>
<td>low- and middle-income countries</td>
</tr>
<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>MAPREC</td>
<td>mutant analysis by polymerase chain reaction and restriction enzyme cleavage</td>
</tr>
<tr>
<td>MCB</td>
<td>master cell bank</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>Middle East respiratory syndrome coronavirus</td>
</tr>
<tr>
<td>MFDS</td>
<td>Ministry of Food and Drug Safety</td>
</tr>
<tr>
<td>MOI</td>
<td>multiplicity of infection</td>
</tr>
<tr>
<td>MPL</td>
<td>monophosphoryl lipid A</td>
</tr>
<tr>
<td>MPS</td>
<td>massively parallel (deep) sequencing</td>
</tr>
<tr>
<td>MRP</td>
<td>minimum release potency</td>
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<tr>
<td>MSL</td>
<td>master seed lot</td>
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<tr>
<td>MVS</td>
<td>master virus seed</td>
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<tr>
<td>NAT</td>
<td>nucleic acid amplification technique</td>
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<tr>
<td>NCL</td>
<td>national control laboratory</td>
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<tr>
<td>NGS</td>
<td>next generation sequencing</td>
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</tbody>
</table>
NIBSC  National Institute for Biological Standards and Control
NIFDC  National Institutes for Food and Drug Control
NIID  National Institute of Infectious Diseases
NLT  not less than
NMR  nuclear magnetic resonance
NMT  not more than
NRA  national regulatory authority
OPV  oral poliomyelitis vaccine
PAGE  polyacrylamide gel electrophoresis
PATH  Program for Appropriate Technology in Health
PCR  polymerase chain reaction
PDL  population doubling level
PEI  Paul-Ehrlich-Institut
PQR  product quality review
PQS  pharmaceutical quality system
PS  polysaccharide
QRM  quality risk management
QM  quality management
R&D  research and development
RBC  red blood cell
RBP  reference biotherapeutic product
rDNA  recombinant DNA
RNA  ribonucleic acid
RRP  recurrent respiratory papillomatosis
SAGE  WHO Strategic Advisory Group of Experts
SBP  similar biotherapeutic product
SDS-PAGE  sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC–HPLC  size-exclusion chromatography – high-performance liquid chromatography
SoGAT  Standardisation of Genome Amplification Techniques (group)
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>SPF</td>
<td>specific pathogen free</td>
</tr>
<tr>
<td>spp.</td>
<td>species</td>
</tr>
<tr>
<td>SSC</td>
<td>Scientific and Standardization Committee (of ISTH)</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TGA</td>
<td>Therapeutic Goods Administration</td>
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<tr>
<td>TNF</td>
<td>tumour necrosis factor</td>
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<tr>
<td>TSE</td>
<td>transmissible spongiform encephalopathy</td>
</tr>
<tr>
<td>USAN</td>
<td>United States Adopted Names program</td>
</tr>
<tr>
<td>vCJD</td>
<td>variant Creutzfeldt-Jakob disease</td>
</tr>
<tr>
<td>VLP</td>
<td>virus-like particle</td>
</tr>
<tr>
<td>VSV</td>
<td>vesicular stomatitis virus</td>
</tr>
<tr>
<td>WCB</td>
<td>working cell bank</td>
</tr>
<tr>
<td>WHOCC</td>
<td>WHO collaborating centre</td>
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<tr>
<td>WSL</td>
<td>working seed lot</td>
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<tr>
<td>WVS</td>
<td>working virus seed</td>
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1. Introduction

The WHO Expert Committee on Biological Standardization met in Geneva from 12 to 16 October 2015. The meeting was opened by Mr Kees De Joncheere, Director of the Department of Essential Medicines and Health Products (EMP).

Mr de Joncheere welcomed the Committee, meeting participants and observers, and reminded the Committee that it had a mandate to review developments in the field of biological substances used in human medicine that included antibiotics, biotherapeutics, blood products, in vitro diagnostic device reagents and vaccines. During its previous 65 meetings the Committee had established approximately 70 written standards and around 300 international biological reference preparations essential for the quality control, regulation and clinical dosing of biological products.

The development and establishment of such standards, and their subsequent promotion, are crucially important activities. The inclusive standards development process implemented by WHO facilitates global consensus on technical matters and is a very significant factor in promoting convergent regulatory decision-making between countries. After standards have been established, proactive technical support from WHO was crucial in obtaining maximum understanding and impact, and facilitating the consistent application of standards.

Equitable access to safe, quality, affordable and effective medical products is one of the cornerstones of universal health care. The development and adoption of norms and standards to regulate the quality, safety, efficacy and cost-effective use of medical products is a vital foundation upon which this aspiration is built. The coordinated efforts of WHO Expert Committees, together with WHO collaborating centres (WHOCCs) and partner organizations, allow for a global approach to this core normative work.

Increased levels of international collaboration are now required in the field of regulatory science. As demands grow for reduced regulatory and policy burdens, expectations of greater transparency will increase. New norms and standards will need to reflect these aspirations but not to the point where quality suffers. As the standards-setting expertise and resources needed to address all needs are likely to be insufficient in any one Member State, the convergence of international norms and standards was increasingly being recognized as a key driver of success. Where appropriate, the use of WHO norms and standards as global benchmarks represents an opportunity to achieve the desired regulatory and policy convergence.

The standardization of biological products remains high on the agenda of Member States and there was now broad recognition of the record of success of biotherapeutic products in treating many life-threatening and chronic diseases. However, some of these products were currently very expensive and access
to them has been relatively limited, particularly in less-resourced countries. This situation was likely to change in the near to mid-term as more countries started to produce biotherapeutics. In addition, the expiry of patents and/or data protection for several originator biotherapeutics had ushered in an era of “similar” products. Nevertheless, many countries also recognize that they do not have the regulatory capacity or expertise to evaluate biotherapeutic products in general and similar biotherapeutic products (SBPs) in particular. WHO will therefore enhance its work with countries to strengthen the capacities of national regulatory authorities (NRAs), quality control laboratories and national pharmacovigilance centres to regulate the safety, efficacy and quality of medical products before their authorization for use, and to monitor their subsequent safety and quality.

Mr de Joncheere reminded the meeting that in May 2014 the World Health Assembly had adopted two resolutions of particular relevance to the above areas, namely: (a) Access to biotherapeutic products including similar biotherapeutic products and ensuring their quality, safety and efficacy; and (b) Regulatory system strengthening for medical products. The progress made in implementing the required actions in these areas requested by Member States will be reported to the Committee, including in the key area of the regulation of blood therapies.

Mr de Joncheere then drew attention to the need to translate the lessons learnt from the parallel development, testing, licensure and use of candidate interventions during the recent response to the complex Ebola epidemic into a “blueprint” for improving research and development readiness for other highly infectious diseases. Biological products were among the most promising candidate interventions for responding to such outbreaks, and the continued strong engagement of the Committee, and the support of all experts and organizations represented at this meeting, was of the utmost importance in such efforts.

Mr de Joncheere pointed out the typically full agenda of the Committee and moved on to the election of meeting officials. In the absence of dissent, Dr Elwyn Griffiths was elected as Chair and Dr John Petricciani as Rapporteur for the plenary sessions, and for the track considering vaccines and biotherapeutics. Dr Harvey Klein was elected as Chair and Dr Clare Morris and Dr Jens Reinhardt as Rapporteurs for the track considering blood products and in vitro diagnostic device reagents. Dr Klein was also elected as Vice-Chair for the plenary sessions of the Committee.

Finally, Mr de Joncheere expressed his thanks on behalf of WHO to the Committee, to WHOCCs, and to all the experts, institutions and professional societies working in this area whose efforts provided vital support to WHO programmes. He concluded by reminding participants that Committee members acted in their personal capacities as experts and not on behalf of their organizations or countries.
The Secretary to the Committee, Dr David Wood, then presented an overview of WHO Expert Committees and of the important and greatly valued role they played in providing assistance to Member States. Established by the World Health Assembly or Executive Board, WHO Expert Committees acted as official advisory bodies to the Director-General of WHO and were governed by formal rules and procedures. Expert Committee meetings were attended by Committee members selected from WHO Expert Advisory Panels; representatives and observers of other organizations including international agencies, nongovernmental organizations and professional associations; Temporary Advisers; and other participants. The reports of the Committee appear in the WHO Technical Report Series and are presented each year to the WHO Executive Board.

Dr Wood then introduced the members of the 2015 Expert Committee and highlighted the new requirement from this meeting onwards that biographical summaries of the members must be posted for public review and comment prior to the meeting. All biographical summaries had been posted and no comments had been received. Dr Wood then outlined the organization of the meeting and the major issues to be discussed. Declarations of Interests made by four members of the Committee, one Temporary Adviser and four participants were then presented to the meeting.\(^1\) Following prior evaluation, WHO had concluded that none of the declarations made constituted a significant conflict of interest, and that the individuals concerned would be allowed to participate fully in the meeting.

Following participant introductions, the Committee adopted the proposed agenda (WHO/BS/2015.2278 Add 2).

\(^1\) Dr E. Griffiths (consulting); Dr P. Minor (public statements and research support); Dr J. Petricciani (consulting and investments); Dr K. Zoon (intellectual property and research support); Dr A. Chawla (travel support); Professor S. Efstahiou (research support); Dr K. Haslov (consulting); Dr S. Inglis (public statements); and Dr S. Thorpe (consulting).
2. General

2.1 Current directions

2.1.1 Strategic directions in biological standardization: WHO priorities

Dr Wood informed the meeting that in order to facilitate coordination of overlapping topics and harness synergies, the Expert Committee on Biological Standardization, the Expert Committee on Specifications for Pharmaceutical Preparations and the International Nonproprietary Names (INN) Expert Group were meeting simultaneously. Dr Wood then outlined the three major categories of outcomes in the area of biological standardization, namely global written standards, global measurement standards and the provision of support to regulatory science including through the development of new assays, further development and refinement of quality control tests, and reviewing of the scientific basis for the establishment of specifications. In addition, standards-implementation workshops continue to be conducted and provide extremely valuable support to participants.

The three global public health priorities of responding to public health emergencies, promoting access to biotherapeutic products and strengthening regulatory systems remain key drivers of strategic approaches in this area. In relation to public health emergencies, an overview from the WHO perspective was presented on the research and development response to the Ebola outbreak. The initial challenge had been to compress the usual timeline for unproven interventions from years to months by working in parallel on product development, testing, licensure and use. From August 2014, WHO held a series of consultations with key international experts and stakeholders to identify potential therapeutic or preventive solutions for Ebola. Based on expert advice, WHO prioritized a number of products for further investigation through human testing, namely two candidate vaccines, a shortlist of biotherapeutic products (including monoclonal antibodies), convalescent whole blood and plasma, and rapid point-of-care diagnostics. By December 2014, clinical trials were under way in the affected countries, with efficacy results now either available or expected for at least one vaccine, six diagnostics and five biotherapeutic products. Experience with the Ebola epidemic and recognition of the vital need for research and development (R&D) preparedness led the Director-General of WHO to conclude that:

...the job now is to harness the lessons from Ebola to create a new R&D framework that can be used for any epidemic-prone disease, in any infectious disease emergency. What is needed is an R&D preparedness plan with clear rules, well-defined platforms for information sharing, and agreed procedures to expedite development and clinical trials.
Next steps will include the preparation of a preliminary outline of a “Blueprint” for R&D preparedness for outbreaks of infectious diseases with epidemic potential, followed by a broad consultative process and submission of the outcome to the 2016 World Health Assembly. Five work-streams and associated deliverables have now been designed to identify the key actions required to accelerate access to products. In relation to the work-stream on the identification of research priorities, the four deliverables selected were: (a) a summary of current status and gaps to be filled to ensure the timely initiation of human trials and introduction of health technologies; (b) an R&D roadmap for Middle East respiratory syndrome caused by a novel coronavirus (MERS-CoV); (c) improved global regulatory preparedness; and (d) a plan for a network of production platforms for priority health technologies.

Progress in relation to the above global health priority of promoting access to biotherapeutic products and reviewing WHO reference preparations in this area is described below in sections 2.1.3 and 2.2.4. In line with the 2014 World Health Assembly, resolution WHA67.21, specific efforts have been made in the areas of biotherapeutic product standards-setting and implementation, as well as in the further development of nomenclature by the INN Expert Group (see section 2.4.1 below). Efforts were also ongoing in the priority area of strengthening regulatory systems, including through the provision of WHO assistance in strengthening national blood systems and supporting the prequalification of in vitro diagnostics (IVDs) (see section 2.1.2 below).

The Committee expressed its support for the focus and priorities outlined for the work of WHO during the coming year.

### 2.1.2 Blood products and related in vitro diagnostics: recent and planned activities in biological standardization

Dr Micha Nuebling presented an overview of activities in a number of core areas including: (a) strengthening local production of blood products; (b) prequalification of antivenoms; (c) prequalification of IVDs; (d) regulation of medical devices; (e) collaborative efforts with WHOCCs; and (f) measurement standards for consideration by the 2015 Expert Committee.

The WHO Achilles project on improving access to safe blood products through local production and technology transfer in blood establishments was then highlighted. This initiative was undertaken in response to resolution WHA63.12 and aims to increase the supply and safety of blood and blood products in low- and middle-income countries by raising standards in blood establishments and reducing the risk of transfusion-transmitted infections. Following the selection of Indonesia as the pilot country, a broad range of project activities had been undertaken and an external assessment had now been made of the extent of implementation of good manufacturing practices (GMP) in a single blood establishment in Surabaya. In light of the progress
made and high level of commitment in this area further activities were now planned. A brief overview was also presented of blood regulation in Kenya and of the collaborative efforts now under way between WHO and the Kenyan National Blood Transfusion Service to improve the safety of the national blood supply. Following up on recent efforts in the WHO African Region, a workshop on the development of a regional strategy for blood safety and the establishment of national regulatory systems for blood and blood products had been held in Benin in September 2015 involving regulators and blood-establishment representatives from 13 countries.

Dr Nuebling then drew attention to the pressing need for the assessment and evaluation of snake antivenoms. With more than 100 000 fatalities occurring as a result of snake bites each year, and the largely unregulated nature of potentially therapeutic products of unknown quality, this continued to be a highly neglected public health issue. As part of its efforts to address this situation WHO was extending its prequalification programme to cover antivenoms. Work was now under way to establish a prioritized prequalification scheme that would be guided by the deliberations of an Expert Review Panel.

The prequalification of IVDs has been an important WHO activity for many years as part of facilitating access to safe, appropriate and affordable IVDs of good quality. Through its standards-setting and guidance-development activities the Committee was requested to further support WHO prequalification efforts in this area.

In light of resolution WHA67.20 on regulatory system strengthening and the paramount requirement for all medical products to be safe and effective, the development of a model regulatory framework for medical devices (including IVDs) was also highlighted as a WHO EMP priority. Against a backdrop of a lack of such regulation in many WHO Member States there was a need for an established process for the approval of medical devices. The development of a first draft of the model regulatory framework was scheduled for completion in early 2016. This would then be followed by a round of public consultation prior to submission of the outcome document for consideration for adoption by the Expert Committees on Biological Standardization and on Specifications for Pharmaceutical Preparations in October 2016.

Dr Nuebling then briefly highlighted the main outcomes areas of the fifth meeting of WHOCCs on the development of WHO biological reference preparations for in vitro diagnostic devices, which are reported upon more fully in section 2.2.2 below. Dr Nuebling concluded by summarizing the programme of measurement standards in the area of blood products and related in vitro diagnostics proposed for establishment by the Committee in 2016, along with the new projects to be endorsed.

There was some discussion around the issue of available resources and expertise to support all the new work areas, particularly the prequalification of
IVDs. In response, it was noted that although the projected activities in relation to the prequalification of IVDs were indeed considerable, these were longer term goals with the level of demands likely to build gradually over the coming years, thus allowing for strengthened collaboration and integration of activities with other processes.

2.1.3 Vaccines and biotherapeutics: recent and planned activities in biological standardization

Dr Ivana Knezevic outlined a range of activities and strategic considerations in relation to the standardization and regulatory evaluation of vaccines and biotherapeutics. To date, a total of 78 WHO Recommendations, Guidelines and guidance documents had been published in these areas. As outlined below in Part 3 of this report, one WHO Recommendations document, two WHO Guidelines documents and one WHO guidance document had been prepared for consideration by the Committee in 2015. In addition, five documents were under development for presentation to the Committee in 2016 with two scheduled for consideration in 2017. One of the documents scheduled for submission to the Committee in 2016 was a revised and updated version of the 2001 WHO Guidelines on clinical evaluation of vaccines: regulatory expectations.2

During the period 2013–2015, eight measurement standards for vaccines had been established by the Committee with a further three expected to be established in 2016. Four workshops on the implementation of vaccine standards had been conducted during 2014–2015, with three planned for 2016 and one for 2017.

In the area of biotherapeutics, six measurement standards had been established during the period 2013–2015, with three scheduled for consideration by the Committee in 2016. Two implementation workshops on biotherapeutics had been conducted in 2014 and two in 2015, with an implementation workshop on the regulatory assessment of biotherapeutic products scheduled for 2016. In addition, an informal consultation on the current WHO Guidelines on SBPs3 had been held in 2015 to assess the need for their revision in view of recent implementation experience. Participants from 26 countries agreed that that there was currently no need for revision but that guidance on monoclonal

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antibody products should be developed as an addendum. An associated consultation on the regulatory assessment of approved biotherapeutic products also recommended that an addendum to current WHO Guidelines in this area\(^4\) be developed and this was one of the written standards scheduled for consideration by the Committee in 2015 (see section 3.2.1 below).

The importance and potential advantages of using case studies and publications during implementation workshops was also highlighted. In addition, the pilot use of interactive e-learning programmes prior to an implementation workshop for WHO guidelines on biotherapeutic products and SBPs had proved to be very helpful in facilitating participant involvement and interaction, with constructive and positive feedback received. Work on the further development of e-learning tools and approaches, and their application in standards implementation, was ongoing.

Projects with timelines yet to be defined included the development of written standards on: (a) respiratory syncytial virus vaccines; (b) meningitis B and meningitis X vaccines; (c) vector-based vaccines; (d) hepatitis E vaccine; and (e) cell and other advanced therapies.

Dr Knezevic then highlighted a number of strategic issues, encompassing the consultation process required for the development of written standards, the development and implementation of written and measurement standards, the need for funding, recent World Health Assembly resolutions, establishing the scope of work and priority setting, improving synergies in vaccine standardization, and the provision of support to global, regional and inter-country networks. In relation to scope and priority setting, Dr Knezevic reported on a proposal for WHO to take the initiative in developing guiding principles for the scientific regulation of cell-therapy products following the 2014 WHA resolution on regulatory system strengthening. It was envisaged that a consultation process may be initiated in 2016 with support from WHOCCs likely to be a key requirement. A number of opportunities for regulatory convergence were also highlighted, including through the collaborative activities of international and regional initiatives, and regulatory and industry networks.

While the recent expansion of the scope of work in all of the above areas had been well received by stakeholders, it was also proving to be very demanding. The Committee agreed that the rate-limiting factor for progress in this area would be the availability of additional resources, with more-efficient ways of

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achieving the goals now required. It was suggested that in view of the recognized capabilities of other organizations that the WHO implementation workload might be reduced through improved collaboration and streamlining of efforts.

2.2 Reports
2.2.1 Report from the WHO Blood Regulators Network
Dr Christian Schaerer reminded the meeting that the objectives of the BRN were: (a) to identify issues and share expertise and information; (b) to promote the science-based convergence of regulatory policy, including by fostering the development of international consensus on regulatory approaches; and (c) to propose solutions to specific issues, especially emerging public health challenges such as the vulnerability of countries to communicable disease threats. As of October 2015 the BRN membership comprised representatives of seven regulatory agencies from different countries and WHO.

Following an update on membership application decisions and other organizational issues Dr Schaerer summarized the range of recent BRN activities. Three teleconferences had taken place in relation to the Ebola outbreak, with further BRN participation in regular discussions on the situation and on activities to strengthen blood systems in affected countries. An expert presentation was given at the Alliance of Blood Operators, and discussions held on their project on risk-based decision-making for blood safety. Discussions were also held on national decision-making in relation to donor deferral for men who have sex with men, and on the potential hazards of transfusion in terms of the increased risks associated with blood transfusions and the postulated late sequelae of transfusion.

In late 2014 the BRN proposed that an amendment be made to its position paper on Collection and use of convalescent plasma or serum as an element in filovirus outbreak response following its posting on the WHO website to include a statement on the use of non-immune plasma as a control in studies with convalescent plasma. In addition, a BRN Letter of support was produced for a request by the World Federation of Hemophilia to add desmopressin to the WHO Model List of Essential Medicines. This request was subsequently accepted at the 2015 meeting of the WHO Expert Committee on the Selection and Use of Essential Medicines.

Following discussions and recommendations made to WHO at the 16th International Conference of Drug Regulatory Authorities (ICDRA) a draft guidance document was developed on the implementation of national blood regulatory systems. BRN representatives also facilitated both the WHO workshop on the development of a regional strategy for blood safety and the WHO workshop on the assessment of GMP in a blood establishment in Surabaya, Indonesia as a part of the WHO Achilles project (see section 2.1.2 above).
Dr Schaerer concluded by highlighting the range of topics that had been identified for discussion by BRN members at its closed meeting during the current session of the Committee.

2.2.2 Report of the fifth meeting of WHO collaborating centres to support the development of WHO biological reference preparations for in vitro diagnostic devices

This meeting was held at the National Institute for Biological Standards and Control (NIBSC) in July 2015 as a follow-up to the previous meeting held in 2011. The meeting objectives were: (a) to coordinate the needs and priorities of individual WHOCCs and WHO programmes; (b) to discuss priority projects in order to avoid the overlap of activities and to strengthen collaboration within the WHOCC network; and (c) to agree on proposals to put forward to the Committee for endorsement or establishment.

Meeting topics had included scientific updates on ongoing projects, an update on relevant WHO guidelines and other guidance documents under development, general discussions on important topics and specific proposals for consideration by the Committee. In their overview presentation to the Committee, Dr Jens Reinhardt and others focused primarily on the scientific updates that had been provided during the meeting as the above WHO documents (on residual risk for virus infections in blood products and on the calibration of secondary standards for IVDs) were dealt with elsewhere in the agenda (see sections 3.3.3 and 3.3.4 respectively) along with an update of developments in WHO prequalification of IVDs (see section 3.3.6 below).

Specific scientific updates provided included a report back from a recent workshop held by the Standardisation of Genome Amplification Techniques (SoGAT) group. This 20th anniversary meeting was held in London in June 2015 and consisted of four sessions over two days on: (a) understanding the use of International Standards and encouraging laboratories to calibrate assays and report results in international units (IU) where an international standard was available; (b) secondary reference materials, with specific focus on their production and calibrated by different manufacturers; (c) validation and calibration of assays in the absence of higher order materials such as international standards; and (d) the standardization of serological international standards, which was an area that had not to date been covered by the SoGAT group. It was agreed that one follow-up activity would be to continue to address serological standardization with invited experts in this field.

A further update was provided on recent developments in hepatitis E virus (HEV) testing and associated clinical and regulatory developments in Europe. The European Pharmacopoeia monograph 1646 had been amended and a paragraph inserted on HEV, noting the need for HEV nucleic acid amplification
technique (NAT)-based testing, with such testing then being implemented in January 2015. This had been preceded by a European Medicines Agency (EMA) workshop on the viral safety of plasma-derived medicinal products with respect to HEV in October 2014. Topics discussed included transfusion-associated infections and related clinical experience, HEV detection and epidemiology of HEV in blood/plasma donations, and approaches to HEV inactivation or reduction. One of the meeting outcomes had been a reflection paper on the viral safety of plasma-derived medicinal products with respect to HEV which had now undergone public consultation.

An update was also given on two CBER research programmes; one on global human immunodeficiency virus (HIV) diversity and one on next generation sequencing (NGS) for pathogens. The first of these projects aimed to create HIV panels consisting of high-titre virus preparations representative of worldwide viral diversity. Fully characterized viruses have now been made available to approved investigators for research purposes and to manufacturers and developers of test kits to validate their assays for detecting emerging HIV variants. At present, 216 high-titre samples from 19 HIV subtypes are available representing the diversity of 29 countries. The use of NSG technology to detect pathogens has broad potential application, including in full-length HIV sequencing, new virus discovery, microbiome and virome characterization of HIV patients and the multiplex detection of bloodborne pathogens.

Significant concerns persist regarding the potential for transfusion-associated transmission of variant Creutzfeldt-Jakob disease (vCJD) and an overview was provided of the two current diagnostic approaches, namely direct detection and amplification-based testing. Both methods were known to present challenges and the meeting was informed that a review had now been conducted by the United Kingdom government science and technology select committee of the progress made in developing a blood test for vCJD. It was recognized that any test to be used for a blood prevalence study should have a demonstrated ability to detect markers of vCJD infection in preclinical animal model samples.

Progress was also outlined in the development of an assay for Ebola convalescent plasma antibodies using a recombinant construct based on vesicular stomatitis virus (VSV) in which VSV glycoprotein (GP) was replaced with Ebola virus GP. Characterization of the resulting construct was described along with the development of two assays based on the above and similar virus constructs for the detection of Ebola antibodies that were now undergoing field validation in West Africa.

An update was then provided on state-of-the-art serological tests for hepatitis C virus (HCV) and for hepatitis B surface antigen (HBsAg), and on HIV antigen/antibody (Ag/Ab) combination assays. For HCV core antigen tests currently on the European market detection limits vary widely. In the area of
HCV antibody testing a switch from indirect enzyme-linked immunosorbent assays (ELISAs) to newer approaches such as “sandwich” ELISA has resulted in increased sensitivity in the diagnostic window phase. Progress had also been made in the development of new HCV Ag/Ab combination tests. Comparative testing of a range of CE-marked HBsAg assays indicated analytical sensitivities ranging from 0.005 to 0.130 IU/ml. For European Commission Common Technical Specifications, the required analytical sensitivity of CE-marked HBsAg screening tests was ≤ 0.130 IU/ml. The development of a new and highly sensitive HBsAg assay was highlighted indicating the feasibility of sensitivity improvements for HBsAg assays. However, HBsAg rapid tests still displayed low sensitivity. For HIV serology, the required analytical sensitivity for p24 antigen detection of at least 2 IU/ml is achieved by the majority of current HIV Ag/Ab combination assays. However, HIV Ag/Ab rapid tests are still not commonly available.

General discussion meeting topics included reported stability issues with established international standards for hepatitis A virus (HAV) RNA, HCV RNA and hepatitis E virus (HEV) RNA. Standards for HCV RNA and HAV RNA had displayed reduced stability during shipping at ambient temperatures and an interim measure had been to send materials on dry ice. Further investigations were ongoing. Although stool-derived (but not plasma-derived) HEV genotype 1 and genotype 2 are unstable (RNA decay) at elevated temperatures following lyophilization in plasma matrix, this effect can be prevented by adding stabilizers.

Dr Reinhardt concluded by presenting an update on proposed actions to be taken with respect to the further development of international standards for a wide range of infectious markers.

2.2.3 Report from the network of WHOCCs for the standardization and evaluation of vaccines

The network of WHOCCs for the standardization and evaluation of vaccines currently consists of eight WHOCCs:

- National Institute for Biological Standards and Control (NIBSC), Medicines and Healthcare Products Regulatory Agency, Potters Bar, the United Kingdom;
- Center for Biologics Evaluation and Research (CBER), Food and Drug Administration, Silver Spring, MD, the USA;
- Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases (NIID), Tokyo, Japan;
- Immunobiology and Biochemistry Group, Office of Laboratories & Scientific Services, Therapeutic Goods Administration (TGA), Woden, Australia;
Dr Junzhi Wang (NIFDC) reported on two symposia that had been organized to discuss recent developments in the quality control and standardization of vaccines. By sharing information on new vaccines, test methods and reference standards a number of new areas for potential collaboration were identified along with opportunities for strengthening collaboration within the network.

An example of the potential benefits of close cooperation between WHOCCs was provided by NIFDC and NIBSC collaboration in the development of an international standard for enterovirus 71 neutralizing antibodies. In this example, leveraging the resources and expertise of two WHOCCs had proved to be highly beneficial in achieving the goal of providing a new international standard for WHO endorsement. Dr Wang went on to outline a range of further successful collaborations between NIFDC and other WHOCCs.

Dr Wang then concluded by describing the key role of regulatory science in Ebola-related activities in China. This had included an emergency two-week quality assessment of an SBP for treating infected patients. In addition, NIFDC had played a central role in Ebola vaccine standardization in China.

A third meeting of the network would be hosted by MFDS in June 2016, and will include a satellite meeting on the evaluation of vaccines for use in a public health emergency. The main focus will be on reaching agreement on priorities, assignment of responsibilities, and the setting of goals and timelines.

The Committee recognized the key importance of collaboration in these and other areas, welcomed the presentation given and requested that it be kept updated of the progress and future activities of the network.

### 2.2.4 Report of a WHO informal consultation on international standards for biotherapeutic products

Dr Adrian Bristow reported on the above consultation, which had been held in Geneva in 2015 in order to review a number of key underlying scientific issues. The consultation had also sought to promote consensus in this area by examining the results of a previously circulated stakeholder survey on various
aspects of the standardization of therapeutic monoclonal antibodies, which had been selected as an exemplar topic for biotherapeutic products in general.

Although the need for such international standards was questioned, the majority view was that these were valuable in: (a) providing a benchmark for biological activity; (b) method development and assessment of system suitability; (c) the calibration of national, pharmacopoeial or in-house reference standards; (d) assessing the potency of multisource products; (e) facilitating product surveillance and life-cycle management; and (f) supporting the development of novel methods.

It was recognized that reference standards are distinct from reference products or reference medicinal products (for biosimilarity) and are not interchangeable, having only limited overlap in both form and function. A reference medicinal product serves to define the quality criteria that a candidate must meet and is used as a comparator in evaluating the biosimilarity of candidate preparations – a function that the reference standard does not serve. Conversely, the reference standard serves to control, define, and calibrate the performance of the test measurement system – a function that the reference biological product cannot serve. In assessing biosimilarity, reference standards thus have a limited but nonetheless essential role. Although such standards do not serve as comparators for defining the quality of an acceptable product they are essential in assessing the suitability of assay method and performance.

Biological activity units are considered to be useful during early stages of SBP development for assay development and qualification purposes but the specific activity of a reference standard should not be used to define acceptable specific activity. In addition, unitage was likely to be specific to different assay classes, and different units for different activities should be assigned. Although the retrospective establishment of a unit should not in principle affect the labelling or dosing regimens of existing or future products, a degree of concern and caution was expressed at the consultation with regard to the unnecessary retroactive applications of units by regulators. At present, there is some degree of polarization of views in relation to the optimal extent of standardization of activity.

In summary, despite recognition of the need for international standards for biotherapeutic products, different views remain across both the regulatory and manufacturing sectors with regard to their precise applications. Other conclusions emerging from the consultation included the need for collaborative studies to always include the innovator product, ideally as a candidate reference standard, but at a minimum for comparison purposes. The maintenance of a global and continuous supply of standards was seen as a key need with the creation of multiple standards not favoured. Where possible, WHO and pharmacopoeial standardization should be coordinated and relevant analytical methods to control method performance developed.
WHO has proceeded cautiously with a standardization programme for monoclonal antibodies and other classes of “new” medicines as they gain marketing authorization through the biosimilar route. There was a need to recognize the concerns and potential impacts for affected stakeholders, and to carefully consider the potential use and extent of applicability of these standards. Consultation participants had therefore urged that WHO consider publicizing the findings of this consultation.

The Committee noted this report, and reaffirmed its support for WHO activities in developing international standards in this area, and ensuring their appropriate use, namely as a benchmark for biological activity, for method development and system-suitability assessment. The Committee also recommended that efforts should be undertaken by WHO to address the polarization of opinion identified in this report and to enhance communication on the appropriate use of such standards. As a first step, areas of agreement should be identified and used as the basis for further stakeholder discussion to further clarify and distinguish scientific and policy issues, and to identify approaches for their resolution.

2.3 Feedback from custodian laboratories

2.3.1 Developments and scientific issues highlighted by custodians of WHO biological reference preparations

The Committee was informed of recent developments and issues identified by the following custodians of WHO biological reference preparations.

National Institute for Biological Standards and Control (NIBSC), Potters Bar, the United Kingdom

Dr Stephen Inglis presented an overview of the standardization activities of NIBSC over the past year. While outlining the NIBSC standardization programme, Dr Inglis highlighted the significant increase in activities and the unprecedented number of projects being brought to the Committee for establishment or endorsement in 2015. Such an expanding level of activity sounded a cautionary note and would necessitate discussion on the specific ways in which the Committee would deal with such a workload going forward. In addition, there were other known areas of standardization that were not yet being addressed by the Committee but which would imminently require attention, such as the application of NGS and genomic testing.

The overview provided also included specific examples of where the institute was moving forward and embarking on new developments within the standardization field. It was noted that the merger with the Medicines and Health Products Regulatory Agency in recent years had allowed for synergies between
the two organizations which had strengthened the position and activities of NIBSC. After detailing a number of completed projects and new proposals for consideration by the Committee in 2015, Dr Inglis briefly outlined the response of NIBSC to the Ebola outbreak, and acknowledged the contribution of all those who had been involved in developing materials for establishment at this meeting in a very short time frame.

Among a number of current broader key issues Dr Inglis pointed out that the expanding field of standardization activities inevitably raised questions concerning the suitability of the current process in flexibly responding to and meeting an increasingly complex range of demands. This raised the issue of whether the current format and associated processes would be sufficiently responsive and sustainable, particularly in the face of resourcing challenges. Dr Inglis also drew attention to the increasing difficulty experienced in publishing research findings in peer-reviewed journal in cases where data had already been presented in the annual report of the Committee as part of the WHO Technical Report Series. This was proving to be restrictive in both conveying standardization messages to the relevant fields and in staff career progression through publication in recognized journals. Dr Inglis then drew attention to a number of upcoming events, which included a symposium on regulatory science in 2016: shaping the future of biological medicines which was being held to mark the 40th anniversary of NIBSC.

Dr Inglis concluded his presentation by announcing that he would be retiring from the position of Director at NIBSC in April 2016 and introduced his successor Dr Christian Schneider.

The Committee joined in thanking Dr Inglis for all his work over the past 14 years and discussed a number of the key issues raised in his presentation. In relation to the specific issue of publication there was some discussion around the use of the journal Biologicals as this peer-reviewed journal was willing to publish material previously presented in the reports of the Committee.

**European Directorate for the Quality of Medicines & HealthCare (EDQM), Strasbourg, France**

Dr Karl-Heinz Buchheit outlined a number of recent EDQM activity areas in biological standardization, including the European Pharmacopoeia, international standards for antibiotics (ISAs), the Official Medicines Control Laboratory network, the European Committee on Blood Transfusion, and in particular its biological standardization programme (BSP) in which WHO has Observer status.

Dr Buchheit reminded the Committee that EDQM was the custodian centre for ISAs – a responsibility that it had taken over from NIBSC in 2006. Since that time, eight ISA replacement batches had been established, with
another proposed for establishment by the Committee this year. As with other international standards, ISAs require distribution, replacement as needed and international collaborative studies. Of the current 23 ISAs eight relate to antibiotics that are on the WHO Model List of Essential Medicines. Dr Buchheit reaffirmed the willingness of EDQM to continue in its role as the custodian centre for ISAs.

BSP goals included the establishment of European Pharmacopoeia biological reference preparations, the standardization of test methods for the quality control of biological substances, the elaboration of alternative methods in support of the 3Rs concept (Replacement, Reduction, Refinement) to minimize the use of animals in research and the provision of support to international harmonization efforts, including through collaboration with WHO and non-European partners. BSP achievements to date included the initiation or conclusion of 102 projects on reference standards and 41 projects on method development (including 21 projects on 3R methods).

Dr Buchheit reiterated that the development of alternatives to animal experiments remained a major commitment of EDQM in line with European Union directives, and WHO was once again strongly urged to consider the incorporation of the 3R initiative into its written standards and other guidance, where appropriate. The inclusion of 3R methods in WHO guidance was viewed by EDQM as being of paramount importance in promoting their global acceptance. Dr Buchheit also requested that the Committee evaluate the possibility of its more proactive and earlier involvement in the validation of 3R alternatives, such as a replacement for the histamine sensitization test for pertussis vaccines. EDQM projects of potential interest to the Committee included the development and evaluation of alternative in vitro tests for both pertussis toxin and pertussis vaccine.

Dr Buchheit also informed the Committee that one of the main outcomes of a recent International Alliance for Biological Standardization conference on 3R alternatives was the decision to formally request WHO to initiate steps to delete the abnormal toxicity test from all WHO Recommendations, Guidelines and other guidance documents. Dr Buchheit went on to suggest that the Committee might begin to consider the implications for IUs for vaccines when in vivo tests are replaced with in vitro tests, and the technical ability to perform the in vivo test has been lost.

Dr Buchheit concluded by highlighting a number of key harmonization and other implementation issues for regional standard-setting bodies when no international standard or other WHO guidance was available. The potential use of international standards and reagents in the veterinary field was also highlighted as a specific issue requiring clarification of the most appropriate lead organization.
Paul-Ehrlich-Institut (PEI), Langen, Germany

Professor Klaus Cichutek presented an update on the recent activities of the two PEI WHOCCs along with an overview of current scientific issues being addressed. Activities of the WHOCC for Quality Assurance of Blood Products and In Vitro Diagnostic Devices had included: (a) development of a WHO Reference Panel for NAT-based assays for HEV genotypes; (b) development of a WHO Reference Panel for anti-HEV antibodies; (c) enlargement of the current WHO repository for transfusion-relevant bacterial strains for platelets; and (d) establishment of a WHO repository for transfusion-relevant bacterial strains for red blood cells.

Other PEI activities included regular and active participation in the BRN and contribution to the development of WHO technical documents on residual risk for virus infections in blood products and on the calibration of secondary standards for IVDs (see sections 3.3.3 and 3.3.4 respectively). Collaboration with the BRN was also undertaken in the important areas of advancing scientific evaluation of the collection and use of convalescent plasma beyond the Ebola outbreak, and ensuring the best use of WHO guidance on assessment criteria for national blood regulatory systems.5

Professor Cichutek then outlined the ongoing PEI support provided to the WHO Achilles project (see section 2.1.2) which had included participation in a range of evaluation workshops and other major project activities requiring the engagement of several PEI experts. PEI was also working to strengthen the blood regulatory systems in two WHO Member States in the WHO African Region.

Activities of the PEI WHOCC for the standardization and evaluation of vaccines during 2014–2015 had included numerous collaborative efforts in the key areas of Ebola, malaria, influenza and dengue. Other activities had included identifying novel strategies for vaccination, discussing potential approaches and requirements for maintaining the emergency availability of smallpox vaccines, and contributing to the development and establishment of WHO guidance on novel vaccines and innovative concepts. PEI also conducted training in the Islamic Republic Iran on the regulation and batch release of vaccines.

A number of current scientific issues were addressed by PEI in the last year in relation to the Ebola outbreak 2014–2015, the seasonal influenza situation,
narcolepsy and MERS-CoV. The Ebola outbreak prompted multiple collaborative research activities involving PEI, including: (a) support for the development of the 2014 BRN position paper on *Collection and use of convalescent plasma or serum as an element in filovirus outbreak response*; and (b) expert participation in the development of an ad hoc Working Group guidance document, updated in April 2015, on the *Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease for transfusion, as an empirical treatment during outbreaks*. PEI also collaborated with the German Center for Infection Research to foster clinical development of the experimental VSV-ZEBOV vaccine and was the responsible German NRA for assessment of the Phase I clinical trials in Africa and Europe.

Data on PEI seasonal influenza activities were then presented, particularly in relation to the now-licensed tetravalent influenza vaccines and the circulation of influenza B strains in the southern hemisphere, and the potential implications for the upcoming northern hemisphere influenza season. An update was also provided on narcolepsy studies in Germany, with one interesting finding being that the incidence of narcolepsy in the country had started to rise prior to the initiation of mass vaccination with the H1N1 influenza vaccine. Professor Cichutek then presented data on a measles-based MERS-CoV vaccine that had shown very promising results in an animal model. The measles-based system for vaccine development was a platform technology that could be adapted to different pathogens, potentially allowing for a rapid response to emerging diseases.

Professor Cichutek concluded by outlining a PEI project on the standardization of birch pollen. Although the significance of pollen allergies has been acknowledged for decades little progress has been made in characterizing the responsible allergens. PEI was investigating the molecular composition of birch allergens in order to allow for their standardization in diagnostic tests and desensitization treatments, and to address the currently high degree of variability in the composition and potency of available products.

*Center for Biologics Evaluation and Research (CBER), Silver Spring, MD, the USA*

Dr Jay Epstein informed the Committee of a wide range of ongoing and proposed activities in the further development of potency standards, reference preparations, international standards, reference panels and reagents.

In response to the Ebola epidemic CBER had expedited the review of investigational new drugs for Ebola vaccine studies and of an investigational device exemption for pathogen-reduced plasma for patient care. Information sharing was facilitated by a Confidentiality Arrangement with WHO, with frequent information exchange and sharing of updates on Ebola-focused activities taking place with WHO, EMA, Health Canada and others. CBER also
participated in the development of physical standards and methodologies for antibody and NAT standards, and for assays for total and neutralizing antibodies. In addition, CBER had participated in African Vaccine Regulatory Forum joint reviews for Phase I and Phase II/III clinical trial protocols for multiple candidate Ebola virus vaccines, which had facilitated the initiation of clinical trials.

Dr Epstein also reported on the CBER-WHO Cooperative agreement to enhance regulatory capacity to support influenza vaccine introduction in low- and middle-income countries (LMIC). Funding had been provided to support NRA assessments, training and an international proficiency study of the single radial immunodiffusion assay. Under another Cooperative agreement, CBER had provided support for the strengthening of global pharmacovigilance capacities for vaccines and, in conjunction with the Uppsala Monitoring Centre, training in the application of pharmacovigilance to regulatory decisions for vaccines.

In 2015, United States Food and Drug Administration approval was given for the first time to an SBP. In addition, a proposed rule and draft guidance was issued on nonproprietary naming conventions for biological products. In the area of blood donation, a final rule on donor eligibility was published in May 2015 that established the requirement to evaluate donors for factors that may adversely affect the safety, purity and potency of blood components or the health of the donor. This final rule also provides a flexible framework for responding to emerging infectious diseases and provides criteria for requiring or removing testing for transfusion-transmitted infections.

CBER personnel also participated in an international study to assess the reproducibility of influenza neuraminidase inhibition titres. This study had been coordinated by the Consortium for the Standardization of Influenza Seroepidemiology and involved 23 laboratories in 12 countries. Factors contributing to assay variability were identified and an enzyme-linked lectin assay protocol will be updated and made available to laboratories working in this area. The development of reference materials for advanced virus detection using NGS was another important new area in which CBER had been cooperatively engaged, and materials should be available by early 2016. Dr Epstein then outlined CBER participation in a number of international studies to develop and evaluate vaccine-related standards for respiratory syncytial virus neutralization assays, human cytomegalovirus immunoglobulin G (IgG), and Ebola antibody. A number of completed and ongoing standards activities for plasma-derived coagulation factors were also described, and current and proposed work on the development of reference preparations for viruses and other pathogens outlined.

Dr Epstein concluded by highlighting a number of recent workshops sponsored by CBER and noting the value of such workshops in the successful implementation of regulatory policy, and the importance of regulatory science research.
2.4 Cross-cutting activities of other WHO committees and groups

2.4.1 Update on matters arising from the International Nonproprietary Names Expert Group

The Committee was provided with an overview of the WHO International Nonproprietary Names (INN) programme and informed that more than 45% of applications for new INN were for biological products. Areas of particular interest to the Committee included the challenges and complexities in relation to the nomenclature used for monoclonal antibodies, recombinant blood products, peptide and recombinant vaccines, and gene-therapy products.

The Committee was reminded that discussions on an INN Expert Group proposal for a Biological Qualifier (BQ) scheme for biological products had been initiated in 2014 with the circulation of a draft proposal for public comment. Following extensive feedback this proposal had been modified and further discussion and feedback had taken place in 2015 resulting in further changes that were to be considered by the INN Expert Group in October 2015. Although the majority of stakeholders supported the use of the BQ scheme, some opposition remained. The Committee was informed that the WHO INN programme will continue to work on modifying the proposal and to propose its implementation at the appropriate time.

The Committee had also been informed in 2014 that consideration continued to be given by the INN Expert Group to the establishment of a nomenclature for cell therapies. The Committee was informed that cell therapies are now approved in a number of different areas of the world, including the United States, Europe and Korea, and that many more such products were reaching the naming stage, with 25 cell therapies in Europe having received orphan designation. The WHO INN programme had received several formal requests for the designation of an INN, and the United States Adopted Names program (USAN) had so far provided over 15 names for cell therapies. Multiple non-uniform naming schemes have the potential to generate great confusion. WHO discussions had taken place over the last 5 years on a naming scheme for cell therapies, with several schemes having been proposed. Difficulties identified included the use of very long and complex names, which would be difficult to memorize and at times pronounce. Such complexity might also lead to transcriptional errors. In addition, as cell-therapy technology rapidly evolves some schemes may not have considered all the possibilities and risk been rapidly outdated. In response to these issues, a working group had been created, stakeholders consulted and a flexible scheme formulated that provided a compromise between the length of the name and the amount of information embedded. The proposed scheme was accepted by both USAN and CBER, and will be discussed during the October 2015 meeting of the INN Expert Group.
In relation to the development of a BQ scheme for biological products the Committee expressed its appreciation for the progress that has been made since it last considered this issue and asked to be kept informed of future developments. It was emphasized that implementation of this scheme should occur soon in order to mitigate the proliferation of national identification schemes. The Committee also welcomed the progress made in the development of an agreed-upon nomenclature for cell therapies and asked to be kept informed of future developments in this area.

2.4.2 Collaborative procedure for facilitating national registration of WHO prequalified medicinal products

The Committee was provided with a brief overview of the potential advantages and key features of WHO prequalification of pharmaceutical products and vaccines. In 2014, the Committee had been informed that a revised procedure to facilitate national registration of WHO prequalified medicinal products was under evaluation and that a revised draft had been prepared and made available for public comment.

Following its endorsement by the Expert Committee on Specifications for Pharmaceutical Preparations, a number of advocacy workshops and capacity-building activities were now being planned to facilitate the use of the revised process. It is intended that a single agreement will be reached with NRAs in participating countries covering both pharmaceutical products and vaccines. The importance of early access to data from manufacturers was stressed as a key issue, particularly in contexts such as pandemic influenza, and the issue of incentives for manufacturers was identified as an area requiring further consideration. The revised procedure remains voluntary for both manufacturers and NRAs, and continues to offer significant potential benefits to both parties. With the agreement of the manufacturer, the full prequalification dossier and site-audit report plus initial testing results would be shared with interested NRAs to facilitate national regulatory decision-making in relation to product registration, variation or withdrawal. The utility of such a collaborative procedure had already been demonstrated, including during the facilitated licensing of inactivated poliomyelitis vaccine (IPV) in several countries.

Discussion was held on a broad range of issues, challenges and opportunities in this area, including in the areas of national and regional regulatory capacity-building, the role of regional implementation workshops and associated initiatives, and the need for manufacturer engagement. The Committee agreed that the revised document would be useful and looked forward to being updated on its implementation at a future meeting.
2.4.3 Proposal to transition from microbiological to physicochemical assays for antibiotic potency testing

There are currently five International Chemical Reference Substances (ICRS) established as secondary reference standards for tests according to chapter 3.1 (Microbiological assay of antibiotics) of The International Pharmacopoeia. To ensure the continuous fitness for purpose of these reference substances, their assigned potencies have to be monitored regularly in extensive and resource-consuming collaborative trials. In addition, a total of 21 monographs prescribe a microbiological assay for antibiotics, but no suitable reference substance has yet been established.

At its meeting in 2009 the Expert Committee on Specifications for Pharmaceutical Preparations had decided that in monographs for antibiotics which specify a microbiological assay, this test should be replaced by a chromatographic method where possible and appropriate. In view of the above, the Secretariat of The International Pharmacopoeia proposed to the Expert Committee on Specifications for Pharmaceutical Preparations and to the Expert Committee on Biological Standardization to:

1. discontinue the use of five ICRS in microbiological assays of antibiotics and to delete the potency assignments in the ICRS leaflets;
2. revise four monographs in order to replace the microbiological assay with liquid chromatography methods, considering methods already published in pharmacopoeias;
3. revise five monographs in order to replace the ICRS with WHO international standards for antibiotics or, preferably, secondary standards derived from them and established by another pharmacopoeia for use in microbiological assays, to foster work-sharing between pharmacopoeias;
4. develop a concept document for the possible transition from microbiological to physicochemical assays in 14 monographs, considering in particular chromatographic methods published in the scientific domain, for discussion and possible endorsement by the above two Expert Committees; and
5. suppress the monographs for substances containing any of five active ingredients – medicines containing these substances are no longer included in the WHO Model List of Essential Medicines (19th List) or in the relevant invitations for expression of interest to manufacturers.
Both Expert Committees agreed to the proposals described in points 1, 2, 4 and 5 above. With regard to the proposal outlined in point 3, it was agreed that the experts should be given more time to identify possible reference standards that could be referred to in each of the monographs. The relevant ICRS and monographs affected by these decisions are listed in Table 1.

Table 1
Recommendations relating to the use of microbiological assays for antibiotics

<table>
<thead>
<tr>
<th>1</th>
<th>ICRS no longer to be used for microbiological assays of antibiotics, and potency assignments to be deleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>nystatin (ICRS0369)</td>
</tr>
<tr>
<td>2.</td>
<td>framycetin sulfate (neomycin B) (ICRS0355)</td>
</tr>
<tr>
<td>3.</td>
<td>gentamicin sulfate (ICRS0319)</td>
</tr>
<tr>
<td>4.</td>
<td>spectinomycin hydrochloride (ICRS0415)</td>
</tr>
<tr>
<td>5.</td>
<td>streptomycin sulfate (ICRS0416)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2</th>
<th>Monographs in which microbiological assay should be replaced by liquid chromatography methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>erythromycin ethylsuccinate</td>
</tr>
<tr>
<td>2.</td>
<td>erythromycin lactobionate</td>
</tr>
<tr>
<td>3.</td>
<td>erythromycin stearate</td>
</tr>
<tr>
<td>4.</td>
<td>tetracycline hydrochloride</td>
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</tbody>
</table>

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<thead>
<tr>
<th>3</th>
<th>Monographs for which suitable standards other than ICRS should be identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>amphotericin B</td>
</tr>
<tr>
<td>2.</td>
<td>amphotericin B for injection</td>
</tr>
<tr>
<td>3.</td>
<td>bleomycin sulfate</td>
</tr>
<tr>
<td>4.</td>
<td>kanamycin for injection</td>
</tr>
<tr>
<td>5.</td>
<td>kanamycin monosulfate</td>
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</table>

<table>
<thead>
<tr>
<th>4</th>
<th>Monographs for which a concept paper should be developed on the possible transition from microbiological to physicochemical methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>amphotericin B</td>
</tr>
<tr>
<td>2.</td>
<td>amphotericin B for injection</td>
</tr>
<tr>
<td>3.</td>
<td>bleomycin sulfate</td>
</tr>
<tr>
<td>4.</td>
<td>erythromycin ethylsuccinate tablets</td>
</tr>
<tr>
<td>5.</td>
<td>erythromycin stearate tablets</td>
</tr>
<tr>
<td>6.</td>
<td>kanamycin acid sulfate</td>
</tr>
<tr>
<td>7.</td>
<td>kanamycin for injection</td>
</tr>
<tr>
<td>8.</td>
<td>kanamycin monosulfate</td>
</tr>
<tr>
<td>9.</td>
<td>nystatin</td>
</tr>
<tr>
<td>10.</td>
<td>nystatin tablets</td>
</tr>
<tr>
<td>11.</td>
<td>gentamicin sulfate</td>
</tr>
<tr>
<td>12.</td>
<td>streptomycin sulfate</td>
</tr>
<tr>
<td>13.</td>
<td>streptomycin for injection</td>
</tr>
<tr>
<td>14.</td>
<td>paromomycin sulfate</td>
</tr>
</tbody>
</table>
Monographs that should be suppressed

1. bacitracin
2. bacitracin zinc
3. bleomycin hydrochloride
4. chlortetracycline hydrochloride
5. erythromycin (base)
6. neomycin sulfate
7. oxytetracycline dehydrate
8. oxytetracycline hydrochloride

Labelling information for influenza vaccines intended for use in pregnant women

The Committee was informed that in 2012 WHO had recommended that pregnant women be vaccinated with trivalent inactivated influenza vaccines (IIVs) at any stage of pregnancy because of the high risk of severe illness and even death associated with influenza in pregnant women and children under 6 months of age. Influenza vaccines have a history of safe use in pregnant women, and the WHO Strategic Advisory Group of Experts (SAGE) on Immunization had requested that WHO develop a plan and associated process for improving maternal immunization coverage. In addition, the GAVI Alliance was considering the inclusion of seasonal influenza vaccination with an emphasis on maternal immunization into its 2014–2020 Vaccine Investment Strategy.

In response to these initiatives, WHO had established a working group to develop advice on labelling information for influenza vaccines intended for use in pregnant women. In July 2014 an informal consultation was held to assess current approaches and experiences. In some countries the wording used in product labels allows for the use of influenza vaccines in pregnant women, as it is not contraindicated. In addition, although precautionary statements may be required by some regulators to reflect the available safety evidence these are not intended to be barriers to use. There is however some diversity among regulators in the use of either cautionary or supportive statements in the package insert which in the case of the former can be misinterpreted by other regulatory authorities, pregnant women, vaccine users and immunization programme managers as a warning against the use of IIVs in pregnant women. It was concluded at the consultation that information should be presented and communicated in a way that would be most helpful to vaccine users, policy-makers, administrators and other relevant parties.

A subsequent survey of Developing Country Vaccine Regulators’ Network (DCVRN) member countries in October 2014 highlighted inconsistencies in the wordings used and information provided in package inserts. In addition, NRAs may have a different interpretation than policy-makers regarding the precise meaning of the wordings used. It was concluded that WHO guidance on
a common policy for package insert wording would improve communication of the benefits and safety of IIVs administered during pregnancy.

A follow-up working group meeting held in September 2015 had reviewed current guiding principles in relation to the vaccine labelling to be used for pregnant women, and had discussed the development and overall issues to be covered in the proposed WHO guidance. It was agreed that such guidance could take the form of an addendum to the existing TRS 927 Annex 3 on IIVs, and that its purpose should be to clarify and assist interpretation of the information provided in the pregnancy subsection of IIV package inserts.

During general discussion a recommendation was made that as the addendum to TRS 927 is developed, due consideration be given to the regulatory principle that data must be available to support conclusions about safety, and that such data are product-specific and usually generated by the manufacturer. The Committee expressed its interest in this project and asked to be kept informed of further progress with a view to possible submission of the addendum document to the Committee in 2016.

### 2.4.5 Influenza vaccine response during the start of a pandemic

The background context for pandemic influenza preparedness and response activities is complex and involves a range of international and other initiatives including the WHO Pandemic Influenza Risk Management framework, the Pandemic Influenza Preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits, and national and regional pandemic preparedness plans.

As part of ongoing WHO efforts in this area the Committee was informed that an informal consultation had been held in June 2015 to discuss and develop specific guidance on initiating an influenza vaccine response during the start of a pandemic. The consultation had involved 33 participants from 18 countries, including representatives from WHOCCs, NRAs, individual manufacturers and manufacturer organizations, academic institutions and other stakeholders. Discussions also focused on identifying the roles and responsibilities of all involved entities.

In addition to the progress made towards the development of a draft operational framework to guide a pandemic vaccine response, key meeting outcomes had included greatly improved understanding of the currently severe timeline constraints and production bottlenecks. At present, 3–4 months appeared to be the minimum time required between the identifying of candidate vaccine viruses and vaccine availability. Maintaining and strengthening the very high degree of global coordination required for the current seasonal influenza vaccine process was also recognized as an essential element in achieving any future improvements in the vaccine development and production timeline.
Potential areas for improvement included: (a) reviewing the current WHO biosafety requirements\(^6\) for manufacturing facilities; (b) reviewing candidate vaccine virus safety-testing standards and release specifications; (c) improving the availability of reagents and assays; (d) ensuring clear and effective regulatory pathways for pandemic vaccines (including for new vaccine platforms and technologies); and (e) improved understanding of the roles and responsibilities of stakeholders in terms of communication and interaction.

During discussions, further clarification was given of the vital importance of promoting and expanding seasonal influenza vaccine use as part of ensuring that sufficient production capacity would exist for pandemic vaccine production. The Committee was also informed of the high degree of interest in moving towards a new paradigm of influenza vaccine virus selection and development that would allow for the more rapid production of well-matched seasonal and pandemic influenza vaccines. The Committee expressed its thanks for the update provided in this important area and asked to be kept informed of further developments.

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3. International Recommendations, Guidelines and other matters related to the manufacture and quality control of biological substances

All WHO Recommendations, Guidelines and guidance documents established at the meeting are listed in Annex 1, which provides an updated listing of all current WHO Recommendations, Guidelines and other documents related to the manufacture, evaluation and quality control of biological substances used in medicine.

3.1 General

3.1.1 WHO good manufacturing practices for biological products

The Committee had been informed in 2014 of the history of WHO good manufacturing practices (GMP) documents for pharmaceuticals, biological products and blood establishments. The most recent WHO guidance on GMP for biological products had been published in 1992 (Annex 2, WHO Technical Report Series, No. 822). Following the initiation of a review process in 2007, a consultation had been held in 2014 with the resulting draft revised Guidelines then undergoing two rounds of public consultation in 2015. The text was further amended by the guidelines drafting group taking into account the comments received.

The outcome revised Guidelines document (WHO/BS/2015.2253) was viewed as complementary to the general recommendations set out in the current WHO good manufacturing practices for pharmaceutical products: main principles\(^7\) and in other WHO documents related specifically to the production and control of biological products. As the revised document does not provide detailed recommendations for specific classes of biological products, attention was drawn in the text to the need to consult other relevant WHO documents, in particular WHO recommendations to assure the quality, safety and efficacy of specific products.

The proposed revision reflects recent scientific and technological developments in the manufacture and control of biological products, and in the application of risk-based approaches to GMP, while recognizing the wide variability inherent in this evolving class of medicinal products.

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The Committee was then provided with a detailed overview of the precise scope and principal themes covered by the revised Guidelines. The document does not cover human whole blood, blood components and plasma-derived products for therapeutic use as separate comprehensive WHO guidance is available and should be followed. The characteristically wide variability typical of biological products and corresponding manufacturing processes, are emphasized in the revised document, along with specific aspects of the analytical methods and approaches required. The importance of in-process controls are emphasized and the quality risk management (QRM) approach recommended as an effective tool for managing product variability, preventing quality deviations and strengthening product and process knowledge.

The Committee reviewed the major comments received during the second round of public consultation and requested a number of clarifications. After making further changes, the Committee recommended that the revised WHO Guidelines be adopted and annexed to its report (Annex 2).

3.1.2 Update on the development of a candidate control material for adventitious virus detection using deep sequencing

The Committee was reminded that in 2010 porcine circovirus-1 DNA had been detected in two rotavirus vaccines using deep sequencing, thus demonstrating the potential utility of this technology in improving or replacing existing adventitious agent tests. However, in order to be useful as a quality control test, the technology must be optimized to determine the most appropriate assay and limits of detection, which in turn requires reference materials to enable the comparison of results and validation of assays.

A reference reagent was formulated by NIBSC (viral multiplex 11/242-001) comprised of a pool of 25 human viruses produced in cells or eggs, or obtained from clinical specimens. Post-formulation qPCR results suggested variable concentrations with six of the viruses being undetectable. The reagent was distributed to 16 laboratories in nine countries for reagent processing using their preferred deep sequencing-based detection method. Most of the laboratories detected at least 20 of the 25 targets with six of the viruses being detected by every laboratory. Two laboratories detected all 25 viruses.

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The study demonstrated that current methods differ in their ability to detect a set of defined viruses. Factors impacting on the ability of a given method to detect an adventitious virus include variable molecular biology, bioinformatics methods, databases and criteria/thresholds for defining a “hit”. The study also underscored the value of a common reagent to enable comparison of different methods.

The reference reagent will be added to the NIBSC catalogue with about 1200 vials remaining, and may be useful as an interim qualitative reference material. Discussions are being held on the design of a new reference reagent containing fewer viruses and at least one prototype from each of the most relevant viral groups. The source of viruses is also under discussion. Discussions of sample requirements and study design are also ongoing with regulatory agencies and other interested parties. It is expected that the new reference reagent will be available in 12–18 months and that a collaborative study will begin in about 12 months.

3.2 Biotherapeutics other than blood products

3.2.1 Regulatory assessment of approved rDNA-derived biotherapeutics

The regulatory assessment of biotherapeutic protein products prepared by recombinant deoxyribonucleic acid (DNA) technology (rDNA-derived biotherapeutics) which, for various reasons, have been licensed with data packages that do not follow current international regulatory standards has been a problematic issue for many countries.

In 2010, ICDRA discussions on such situations were held and WHO requested to assist in developing approaches for evaluating these already-licensed products according to current WHO guidance. In 2014, the Sixty-seventh World Health Assembly adopted two relevant resolutions: one on promoting access to biotherapeutic products and ensuring their quality, safety and efficacy, and the other on regulatory systems strengthening in which WHO was requested to provide guidance, especially on dealing with increasingly complex biological products.

The purpose of the proposed addendum document\(^{10}\) was to provide guidance on the regulatory assessment of rDNA-derived biotherapeutics in cases where their licensing did not follow current international regulatory standards – including, for example, biotherapeutic products licensed via a generic pathway or with limited analytical, nonclinical and/or clinical data.

Although dealing primarily with rDNA-derived biotherapeutics, some aspects of the document may also be relevant to other biotherapeutics.

Specific areas addressed included regulatory expectations for rDNA-derived biotherapeutics (including SBPs), the reviewing of products already on the market, the points to consider in a stepwise regulatory assessment and the regulatory actions to be considered following assessment. The stepwise regulatory assessment approach outlined was designed to offer flexibility and to promote the accessibility of biotherapeutic products of assured quality, safety and efficacy.

The outcome document (WHO/BS/2015.2251) had undergone extensive consultation involving six rounds of regulatory expert review and three rounds of public consultation. Participation in the consultation process had been broad and had involved NRAs and national control laboratories (NCLs) from 41 countries in all six WHO regions, EMA and DCVRN, three pharmacopoeias, 11 manufacturer associations and 14 individual manufacturers in nine countries.

The Committee reviewed the document and after proposing a number of changes to further improve the clarity of the text, recommended that the proposed addendum be adopted and annexed to its report (Annex 3). The Committee also emphasized the urgent need for implementation workshops in this area.

### Blood products and related substances

#### Ebola-related issues

The Committee was provided with an overview of concerted efforts made by WHO to prioritize the further investigation of a number of products for the prevention or treatment of Ebola infection. Three field studies on the use of convalescent plasma had been conducted in Guinea, Liberia and Sierra Leone. Differences and similarities in the approaches taken were highlighted and acknowledgement made of the high level of teamwork demonstrated by the multiple organizations involved. Final study results from the largest of these studies, conducted in Guinea and constituting the largest ever trial of convalescent plasma, were expected in the coming weeks.

In early 2015 a meeting of directors of national blood transfusion services was held and a range of views expressed in relation to the conducting of the trials and the challenges faced. Given the extent of pre-existing health system weaknesses in each of the above affected countries the achievement was especially notable. One key aim now was to catalyse the short-term support for research and development efforts in the blood area into medium- and longer-term sustainable support for the development of the national blood systems. It was then highlighted to the Committee that while the response to the 2014/2015 Ebola epidemic had progressed well in a short time frame, there was a need for a
much more rapid response, and that lessons had been learned that would assist in defining rapid-response requirements for potential future pathogen outbreaks.

The Committee observed that there had been some degree of criticism following a perceived slow response from WHO. Nevertheless, it was also apparent that the scale of work and progress made in a very short space of time had been considerable.

3.3.2 Local production of blood products

An overview was provided to the Committee of WHO activities conducted in Indonesia, Kenya and Benin in relation to the strengthening of local production of blood products. Indonesia had been chosen as a pilot country for the WHO Achilles project on fostering the use of recovered plasma to produce GMP-compliant plasma products. This project started in 2013 with a situation analysis of the national regulatory framework, which was followed in 2014 by workshops on the evaluation of screening tests and GMP training. In 2015, an expert assessment was made of GMP in a selected pilot blood establishment in Surabaya. It was concluded that the establishment had excellent starting conditions, being a successful blood service with a high donor-recruitment rate and good infrastructure. Moreover, a quality management (QM) system had been introduced, albeit to varying degrees in different departments. Recommendations from the assessment included strengthening of the QM system and liaising with a blood regulatory system in the geographical region. In continuation of the project planned activities included: (a) holding a workshop with the Ministry of Health reference laboratory for blood screening and diagnostic IVDs, covering the assessment of manufacturer performance data, evaluation algorithms and cooperation with established reference laboratories; and (b) reassessment of GMP implementation, potentially again in Surabaya following initiation of the use of plasma for fractionation.

In Kenya, an assessment of the blood regulatory system was conducted in 2015. The Committee was provided with a brief overview of the status of blood regulation in Kenya, the significant challenges still being faced, and the initiation of collaborative efforts between WHO and the Kenyan National Blood Transfusion Service to improve the safety of the national blood supply. Following up on recent efforts in the WHO African Region, a workshop on the development of a regional strategy for blood safety and the establishment of national regulatory systems for blood and blood products had been held in Benin in 2015 involving regulators and blood-establishment representatives from 13 countries, as well as representatives from WHO, WHO BRN and PEI. This workshop had provided an opportunity for the sharing of national experiences in blood regulatory activities, and for the drafting of a regional blood safety strategy for the upcoming 10 years along with guidelines on the establishing of a regulatory system for blood and blood products.
During discussion, it was clarified that Indonesia intends to initiate plasma fractionation within the next two to three years. The Committee was also informed of an upcoming International Society of Blood Transfusion meeting in November 2015 at which an update on the national blood system in Indonesia will be presented. The Committee concluded that WHO initiatives in this area had been successful and should be continued. However, it was also acknowledged that resourcing and capacity issues would likely arise as levels of demand for WHO assistance increased.

3.3.3 Residual risk of virus infections caused by blood products

An outline was presented to the Committee of draft WHO Guidelines on estimating the residual risk of virus infections caused by blood products (such as cellular blood components or recovered plasma) obtained from whole blood donations. The development of such guidance had been requested by blood-donation centres in LMIC during meetings held in the context of the WHO Achilles project. It had been recognized that currently there is no common and easy approach for calculating the residual risk because of different screening assays used in different regions, and a corresponding lack of data on both assay characteristics and blood-donor epidemiology.

In June 2015, a WHO Working Group meeting had been convened and draft guidelines prepared to differentiate between screening-test categories and define the mean diagnostic window phase for each category. Furthermore, maximal viral loads during the diagnostic windows of different virus infections (HIV, HBV and HCV) were defined. Combined with epidemiological data on the donor populations an approach for the calculation of residual risk was proposed. For repeat-donor subpopulations, residual risk estimation is primarily based upon the infection rate (incidence) based upon the number of seroconversions in a one-year observation period, donation frequency and length of the viraemic window phase. In first-time donor subpopulations incidence is currently typically determined using more complex approaches such as modified antibody assays or NAT-only data.

In the draft Guidelines under development it has been proposed that incidence in this latter subpopulation may be reflected by the incidence in the respective repeat-donor subpopulation combined with an adjustment factor. Further guidance includes an adjustment factor for the transient early markers of HBV infection and a formula for the calculation of maximal numbers of window-phase donations. Using the proposed approach may allow for greater consistency in calculation methods, thus allowing for improved comparisons between different donor populations and different blood centres. It is expected that the Guidelines will also facilitate decisions on screening-test strategies based on cost–benefit analyses, and help plasma fractionators to decide upon the acceptability of recovered plasma.
Discussion focused upon the acceptability of using the proposed extrapolation factor of x3 to estimate incidence in first-time donors based upon incidence among repeat donors. However, since this adjustment represents a worst-case scenario and given that further testing would still be possible at later stages, it was considered acceptable. A further issue raised was the extent to which the accuracy of IVDs was precisely known and the potential need for additional cautionary text on this in the proposed document.

3.3.4 WHO general assessment tool for conducting gap analysis of national regulatory systems for health products and technologies

An overview was presented of a proposed WHO general assessment tool for conducting gap analysis of national regulatory systems for health products and technologies. The development of this tool had been agreed upon at the January 2015 WHO International Consultation on Regulatory Systems Strengthening. The purpose of the general assessment tool was to allow for harmonization of the broad range of different assessment tools currently used for different product streams and/or technologies by both WHO and non-WHO agencies, and to permit the harnessing of synergies between already existing assessment systems. One such example was the recently adopted WHO assessment criteria for national blood regulatory systems.\(^{11}\) It was envisaged that use of the general assessment tool and associated alignment of tools used for individual product areas would help ensure the compatibility of assessments or other evaluations, thus avoiding the need to repeat work already done.

The tool had been discussed during the 2015 consultation and developed by WHO on the basis of the advice received. The current version of the tool was outlined to the Committee for its information and would be subject to another round of international consultation in December 2015 with further progress to be reported to the Committee in 2016.

During discussion, it was pointed out that while such a general assessment tool may be very useful, and that amendment of the WHO assessment criteria for national blood regulatory systems would potentially be beneficial in principle, it would also be important to preserve the details of the different product streams and/or technologies addressed by individual assessments. It was also reiterated that the assessments resulting from already existing tools would need to be accepted after validation to avoid duplication of efforts. It was further pointed out that the terminology used for the different categories of product needed to

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be consistent. The Committee recommended that the topic be further discussed at the next BRN meeting.

3.4 In vitro diagnostic device reagents

3.4.1 Calibration of secondary standards for in vitro diagnostic devices

The development of a WHO guidance document outlining principles for the calibration of secondary reference materials for IVDs was first discussed in July 2012 and endorsed by the ECBS in October that year. The proposed document was intended to provide practical guidance on the preparation of secondary references and their calibration relative to WHO international standards, facilitate a consistent approach to calibration, improve the availability of secondary reference materials and contribute to the global harmonization and quality assurance of IVDs.

The document is intended for use by all manufacturers of secondary references, and covers both NAT-based and serological infectious disease assays, the general principles of biological standardization, considerations in the preparation of secondary references, and the need for collaborative studies, instructions for use and eventual replacement.

Following collaborative preparation of the first draft by NIBSC and PEI, the document was presented at the 20th anniversary meeting of the SoGAT group. Meeting participants provided comments and suggested that a number of other organizations not present at the meeting should be made aware of the document. The resulting revised draft combined all comments received to date and it was now the intention to seek the input of the Committee prior to the completion of a final draft version for submission to the Committee in 2016.

During discussion, the issue of overlapping WHO guidance was raised and it was highlighted that the current WHO Guidelines outlining the development of an international standard already makes general mention of the need for appropriate calibration of secondary reference materials. In response, it was suggested that the proposed document was required to provide more detailed and specific information to address needs in this area. Discussion then continued in relation to the precise scope and placement of the proposed document in the context of existing WHO guidance. It was agreed that such decisions would be driven largely by whether the document was to be incorporated into the overview guidance or to remain standalone.

Discussion was then held on the envisaged main users of the document. Although there were commercial manufacturers of such materials the restricted supply does not meet the needs of assay developers, and the area would likely benefit from a harmonized approach to the production of these materials. It was noted that smaller NCLs may also benefit from such a resource, particularly where they had ready access to patient materials that could serve as a standard.
After further consideration it was decided that a final version of the document should be presented to the Committee in 2016 prior to a decision being made on its placement in the context of current guidance.

3.4.2 WHO prequalification of in vitro diagnostic devices

The WHO Prequalification Team is part of WHO/EMP and provides independent technical information on the safety, quality and performance of IVDs. While certain IVDs (for example, those used to aid in the diagnosis of HIV infection) have been assessed by WHO since 1998, a shift occurred in 2008 away from assessments based solely on performance evaluation and towards a more robust prequalification assessment which also includes dossier review and manufacturing site inspection. Future planned developments of the IVD programme were intended to lead to the development of a set of guidance documents and the conducting of training sessions to assist IVD manufacturers.

Many of the IVDs used in resource-limited settings were rapid diagnosis tests, with prequalification dossier reviews indicating that many manufacturers did not properly use existing reference materials. Independent lot testing at product delivery to countries was also recommended for such products to ensure that performance had not been jeopardized during transportation and storage. It had been concluded that for IVDs used for priority diseases, reference materials that cover all genotypes and subtypes, as well as common mutants, are needed. Also, clear regulatory requirements on the use of reference materials need to be developed, with clear indications that such materials also need to be commutable and fit for purpose for use outside Europe and the United States.

The Committee congratulated the group on the breadth of this important work. In the longer term this breadth may raise issues in relation to the available expertise within current collaborating centres. The Committee was informed that that the Prequalification Team regularly interacted with other WHO work programmes to assess priority needs, and it was acknowledged that linking the prequalification of IVDs to the Committee process would also assist in the prioritization of standardization projects. However, it was also acknowledged that sourcing sufficient funding may present a problem in the continuation of its work. Discussion also took place of the approach of the Committee in establishing one international standard for each analytical marker, with subsequent reference materials for that marker comprising a defined reference panel. Such an approach may prove to be too restrictive for prequalification needs.

It was noted that the proposed further development of guidance documents was needed as there was a lack of knowledge regarding available materials (for example, secondary standards) and of their calibration and correct use. Data exchange was also discussed and acknowledged to be another area
where guidance and development work is needed. After further consideration it was proposed that the Prequalification Team develop guidance for manufacturers on the use of international reference materials for the calibration and characterization of diagnostic assays with a view to presenting this to the Committee in 2016.

3.5 Vaccines and related substances

3.5.1 Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines

WHO Guidelines to assure the quality, safety and efficacy of recombinant human papillomavirus (HPV) virus-like particle (VLP) vaccines were first adopted by the Committee in 2006\(^\text{12}\) and were based largely on experience gained from clinical trials undertaken on the first two licensed HPV vaccines.

Factors that prompted the revision of these Guidelines included the substantial amount of data accumulated during vaccine implementation, the development of prophylactic vaccines with extended valency and the use of other production methods. In addition, the increasing availability and routine use of HPV VLP vaccines composed of L1 capsid protein and containing at least types 16 and 18 have important implications for trial designs and end-points for clinical evaluation of new prophylactic HPV vaccines.

In 2013, a series of meetings was convened by WHO to review new HPV vaccines under development, and to discuss the evidence and scientific basis for accepting alternative end-points for evaluating the clinical efficacy of candidate HPV vaccines. In 2014, a drafting group meeting and informal consultation were held to draft, review and further develop revised WHO Recommendations in this area. The informal consultation was attended by experts from around the world involved in the research, manufacture, licensing/authorization, control-testing and release of HPV vaccines, with participants drawn from academia, NRAs, NCLs and industry. Two rounds of public consultation on the outcome text were then held.

Major issues addressed in the revised WHO Recommendations included:

- updating of terminology;
- updating of general considerations and other sections to reflect the current development of HPV vaccines;

updated section on international standards and reference preparations;
updated Part A in order to include bacteria as a cell substrate;
updated Part B in order to include a new subsection highlighting tests needed for nonclinical evaluation, and to elaborate regulatory requirements for both proof-of-concept pharmacology studies and toxicological testing;
updated Part C in order to reflect appropriate and feasible end-points in future trials;
updating of appendices.

Additional changes had also been made to bring the revised document (WHO/BS/2015.2252) into line with other WHO Recommendations, Guidelines and guidance documents published since the 2006 adoption of the WHO Guidelines on recombinant HPV VLP vaccines.

The Committee was informed of the key issues addressed and specific approaches taken in each of the major sections of the revised document, and provided with a summary of the comments and other inputs received during the rounds of public consultation. After carefully reviewing the suggested approaches and comments presented, and making a number of further changes, the Committee recommended that the revised WHO Recommendations be adopted and annexed to its report (Annex 4).

3.5.2 Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions

Vaccines are complex biological products and may undergo degradation during long-term storage under cold chain conditions (for example, 2–8 °C) and this is typically enhanced at higher temperatures. Consequently, establishing the stability characteristics of products is a critical element of the overall evaluation by an NRA to ensure that licensed vaccines remain efficacious at the end of their shelf-life when stored under the approved conditions.

In response to the stability assessment needs identified by NRAs, WHO developed guidelines on the stability evaluation of vaccines. While it was well understood that vaccine quality depends on cold chain storage, it was also recognized that immunization programmes in certain regions faced substantial challenges in maintaining cold chains in the field, especially during the final stage of distribution in remote areas. To address these distribution challenges and expand immunization programmes into specific regions WHO developed a “controlled temperature chain” (CTC) programme. This programme currently requires that a vaccine exhibits a stability profile suitable for a single exposure to at least 40 °C for a minimum of 3 days just prior to administration, while
remaining compliant with the approved vaccine specifications. Additionally, the programme requires that the CTC provision should be included in the licensure by the relevant NRA and by WHO prequalification.

The term “extended controlled temperature conditions” (ECTC) had been introduced to distinguish regulatory requirements from WHO CTC programme aspects. ECTC assessment establishes the short-term performance of a vaccine at any temperature above those of a typical cold chain, independent of the specific programmatic requirements of the current WHO CTC programme. Vaccines licensed for use under ECTC are required to have sufficient information regarding the approved conditions (such as maximum temperature and time) on the package insert.

The proposed WHO Guidelines (WHO/BS/2015.2268) arose from WHO immunization programme requirements and from discussions held by an international group of vaccine-stability experts at two WHO-sponsored consultations – held in Ottawa, Canada in December 2012, and Langen, Germany in June 2013. The Guidelines are intended to supplement the broader WHO Guidelines on stability evaluation of vaccines and focus on ECTC-specific issues not covered in existing guidance with as little overlap as possible. Key elements include the application of current mathematical modelling and statistical concepts to the unique short-term requirements that apply to some cases of vaccine distribution and use. A product-specific ECTC evaluation of a model monovalent polysaccharide conjugate vaccine is also provided as a case study.

The Committee reviewed the document and the key issues that had been raised during the consultations and, after making a number of changes, recommended that the WHO Guidelines be adopted and annexed to its report (Annex 5).

3.5.3 Vaccine evaluation in public health emergencies – review of regulatory pathways in selected countries

The Committee was informed of various WHO initiatives and activities related to the use of vaccines in public health emergencies. The development and licensing of vaccines against emerging infections that are declared a public health emergency involved numerous challenges, including severely contracted timelines. Of particular importance was the recognition that regulatory processes needed to be in place to enable the rapid evaluation of submissions.

and – following careful risk–benefit assessment – to allow the use of vaccines for which a full regulatory package may not yet be available.

Following an informal consultation in 2015 and subsequent survey feedback received from 10 NRAs, WHO had now developed a review document which aimed to provide an overview of various existing regulatory pathways in selected countries and to encourage countries to review their state of regulatory preparedness for public health emergencies. Experience of public health emergencies since 2000 included the severe acute respiratory syndrome outbreak in several countries, the H1N1 influenza pandemic (and ongoing risk of an avian influenza pandemic) and the Ebola epidemic in West Africa. Each of those events served to reinforce the crucial need for a rapid global response.

It was determined by WHO that a certain level of flexibility already exists in some countries in relation to the evaluating of vaccines during public health emergencies. However, there were also countries in which vaccine licensing could only be done on the basis of a full data package with no alternative pathway. In such cases, it would be important to initiate discussion at national and regional (or intercountry) level to explore options for establishing alternative regulatory pathways as part of overall emergency response efforts.

The most important considerations in evaluating vaccines during public health emergencies included risk–benefit assessments, the need for a well-defined and transparent decision-making process, risk-management planning and pharmacovigilance. It had been further concluded that actual case studies and lessons learnt from previous public health emergencies could serve as training materials.

The Committee noted the draft review document and sought clarifications in a number of areas. While it was agreed that a WHO guidance document might be of particular value to some NRAs and public health organizations, there was also recognition of the complexity of emergency situations. The decision to approve the use of an investigational product in the event of a public health emergency would ultimately rest on a case-by-case risk–benefit assessment. The Committee looked forward to reviewing the progress made in the further development of the document in 2016.

3.5.4 Development of Guidelines on the quality, safety and efficacy of Ebola vaccines

Following the start of the Ebola epidemic in West Africa in 2014, considerable efforts have been made in relation to the development, evaluation and licensing of Ebola vaccines. The Committee was informed that as part of ongoing WHO measures to support the development of Ebola vaccines a draft concept paper had been prepared which had outlined the scientific and regulatory considerations in the evaluation of vaccines intended for emergency use, and
which had included related guidance regarding the quality, safety and efficacy of Ebola vaccines.

Following expert review, the need for a specific Ebola vaccine document was highlighted. In March 2015, WHO convened an informal consultation attended by experts, regulatory professionals and other stakeholders involved in Ebola vaccine development, production, and regulatory evaluation to review the resulting draft WHO Guidelines and reach consensus on key technical and regulatory issues.

The draft Guidelines were intended to provide guidance to NRAs and vaccine manufacturers on the quality, nonclinical and clinical aspects of Ebola vaccines, particularly those based on viral vectors as these are currently at the most advanced stage of development.

As there are currently significant knowledge gaps in the scientific understanding of Ebola disease and Ebola vaccines, and as no Ebola vaccine has yet been licensed, the document had been developed as WHO Guidelines rather than Recommendations. It was recognized that subsequent updating of the Guidelines may be required in the light of recent and future developments.

The Committee noted the comments and suggestions that had been received by WHO in relation to the draft Guidelines and indicated its agreement with the proposed content, scope and proposed approach for its further revision. The Committee also provided a number of additional recommendations, including the addition of further guidance on post-marketing surveillance and the need for fluid approaches to updating and posting the current text in this rapidly evolving area. The Committee expressed its appreciation for the efforts that had been made in developing such an excellent and comprehensive draft in such a short period, and looked forward to considering the revised Guidelines at its next meeting.

3.5.5 Revision of Guidelines on the safe production and quality control of inactivated poliomyelitis vaccines manufactured from wild polioviruses

The Committee was reminded that the WHO Polio Eradication and Endgame Strategic Plan 2013–2018 (PEESP) published by the Global Polio Eradication Initiative sets the goal of achieving a polio-free world by 2018. Towards this goal, activities include minimizing the risk of facility-associated reintroduction of wild or attenuated oral poliomyelitis vaccine (OPV, Sabin) polioviruses. In the now finalized third edition of the WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) published in May 2015, the safe handling and containment of poliovirus infectious and potentially infectious materials has been aligned with the PEESP. WHO was currently developing a supplementary document – GAPIII Containment Certification Scheme – to
help facilities and oversight bodies better understand and implement GAPIII requirements as they relate to the certification of poliovirus containment in essential facilities.

The Committee was informed that current WHO Guidelines for the safe production and quality control of IPV manufactured from wild polioviruses had been published as an addendum to the previous WHO Recommendations for the production and control of poliomyelitis vaccine (inactivated). In light of a range of associated developments and updates, including in the area of GMP for biological products, the revision of the current Guidelines had been proposed.

In order to inform the revision process and identify key issues to be addressed, a thorough expert technical review was conducted involving WHOCCs working in this area. The need for revision of the current guidance was broadly recognized in order to provide practical and detailed guidance to poliomyelitis vaccine manufacturers and regulators not fully addressed by any currently available WHO documents. Ideally, the revision would extend the scope of the current document to cover all poliovirus strains, and provide updated, detailed, specific, and practical guidance on all aspects of poliovirus containment in vaccine-manufacturing facilities, with an emphasis on issues not addressed elsewhere. The revision would also take into account the current and anticipated future global polio eradication situation and be aligned with current resources and global strategies such as PEESP and GAPIII. Further details and specific issues will be identified and discussed during consultations with experts from vaccine manufacturers and NRAs.

The Committee noted the information presented, sought clarifications in a number of areas and made several specific suggestions. The Committee agreed with the conclusions and proposals presented, expressed its support for the development of the revised WHO Guidelines and looked forward to reviewing progress in 2016.

3.5.6 International collaborative study to assess the utility of deep sequencing in the quality control of oral poliomyelitis vaccines and inactivated poliomyelitis vaccines made from Sabin strains

An international collaborative study on the utility of massively parallel (deep) sequencing (MPS) in monitoring the molecular consistency of OPV had been endorsed by the Committee in 2013. Preliminary data were subsequently collected in 2014, and a workshop convened in April 2015 attended by NRAs, NCLs and poliomyelitis vaccine manufacturers from several countries.

The status of OPV quality control in different countries was reviewed and the experiences of several groups in using MPS shared. After discussing the objectives and design of the proposed collaborative study, workshop participants concluded that Phase 1 of the study should focus on the evaluation of MPS
analysis as a potential replacement for the mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) test. Phase 2 of the study would then concentrate on generating whole-genome sequence polymorphism profiles for use in assessing the consistency of newly prepared vaccine batches when compared with previously released vaccine lots.

The Committee was provided with an overview on the objectives, design and justification for each proposed phase, and updated on the current status of the study. The Committee noted the proposals made in relation to each of the two phases, sought clarifications in a number of areas and made several specific suggestions. The Committee agreed that the study would provide useful information, and looked forward to reviewing progress in 2016.
4. International Reference Materials – antibiotics

All reference materials established at the meeting are listed in Annex 6.

4.1 WHO International Standards and Reference Reagents – antibiotics

4.1.1 Fourth WHO International Standard for streptomycin

Streptomycin is an aminoglycoside antibiotic used in the treatment of tuberculosis and of moderate to severe susceptible infections, including plague, tularemia and bacterial endocarditis. It is also used as second-line therapy for gram-negative bacillary bacteraemia, meningitis, pneumonitis, brucellosis, granuloma inguinale, chancroid and urinary tract infections. Streptomycin appears on the WHO Model List of Essential Medicines.

The Third WHO International Standard for streptomycin was established in 1980 on the basis of an international collaborative study and was assigned a value of 78 500 IU/ampoule. As stocks of this international standard were dwindling, appropriate steps were taken by EDQM for its replacement.

An international collaborative study was conducted to establish the Fourth WHO International Standard for streptomycin. Thirteen laboratories from 12 countries participated. The biological activity of the candidate material (EDQM code 55821) was estimated by microbiological assays using sensitive microorganisms. To ensure continuity between consecutive batches, the current international standard was used as a reference.

Stability studies demonstrated that the candidate material was stable at temperatures used for storage (−20°C). The results of accelerated thermal degradation studies at 20°C, 37°C and 45°C for 1, 3 and 6 months indicated that the candidate material was stable for long-term use. Real-time stability studies were now in progress. The Committee was informed that 993 ampoules would be available for use as an international standard.

The Committee considered the report of the study (WHO/BS/2015.2277) and recommended that the candidate material 55821 be established as the Fourth WHO International Standard for streptomycin with an assigned value of 76 000 IU/ampoule.
5. International reference materials – biotherapeutics other than blood products

All reference materials established at the meeting are listed in Annex 6.

5.1 WHO International Standards and Reference Reagents – biotherapeutics other than blood products

5.1.1 First WHO International Standard for tumour necrosis factor receptor Fc fusion protein (etanercept)

The recombinant human tumour necrosis factor (TNF) receptor Fc fusion protein (etanercept) is a large glycoprotein with a molecular weight of approximately 150 kilodaltons. It acts as a competitive inhibitor of TNF and prevents it from binding to its cell surface receptors, thereby reducing the biological activity of TNF. As a result, it has potential application in the treatment of various autoimmune diseases or disorders associated with increased TNF and excess inflammation. Current therapeutic indications include rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis and plaque psoriasis.

Several versions of etanercept are already approved in some countries with a number of SBPs now undergoing regulatory evaluation or clinical trials in various other countries. Etanercept products are dosed in mass units and current labelling does not provide information on biological activity (that is, in terms of international units or specific activity of the protein). In vitro determination of biological activity against proprietary reference materials is routinely performed by license holders for lot release and stability assessment. The development of a publically available international standard would facilitate determination of the biological activity of intended copies or potential SBPs, thus ensuring patient access to products of consistent quality and effectiveness. This project had been endorsed by the Committee in 2012.

An international collaborative study was conducted to establish the First WHO International Standard for tumour necrosis factor receptor Fc fusion protein (etanercept). Three candidate samples were provided by two different manufacturers and were analysed by 28 laboratories from 15 countries using their own in-house bioassay. Within- and between-laboratory repeatability was generally very high with excellent agreement observed between laboratories regardless of which candidate was chosen as the standard for determining sample potency. Two of the candidate samples (13/192 and 13/204) were found to be suitable for use as an international standard.

Stability studies demonstrated that the above two candidate materials were stable at temperatures used for storage (−70 °C) and for laboratory manipulation (4–20 °C). The results of accelerated thermal degradation studies
at 14 to 19 months indicated that both candidates would be stable for long-term use. Real-time stability studies were now in progress. The Committee was informed that 4700 ampoules would be available for use as an international standard.

The Committee considered the report of the study (WHO/BS/2015.2257), made several suggestions on the information to be included in the Instructions for Use and recommended that candidate material 13/204 be established as the First WHO International Standard for tumour necrosis factor receptor Fc fusion protein (etanercept) with an assigned value of 10 000 IU/ampoule.

5.1.2 First WHO Reference Panel for antibodies to erythropoietin (human)

Recombinant human erythropoietin (EPO) has been approved for the treatment of anaemia with subsequent approval obtained for several other erythropoiesis-stimulating agents (ESAs) worldwide. Currently, a number of different EPO products of markedly variable quality are marketed worldwide. Despite their successful use, EPO products can in rare cases induce a potentially life-threatening immune response in recipients. Evidence to date has shown that human EPO induces neutralizing antibodies which not only cross-react with all EPO products but also neutralize the endogenously produced protein causing antibody-mediated pure red cell aplasia (PRCA). Isolated PRCA cases have also been linked to the use of other ESAs.

There are no universally accepted methods or reference reagents for detecting and measuring EPO antibodies. However, several methods which differ in the types of antibodies detected and in sensitivity are used to detect antibodies against EPO. The availability of reference standards/reagents for the testing of EPO antibodies across different assay platforms would allow for consistency in the detecting and measuring of EPO antibodies as part of monitoring the safety and efficacy of ESAs. Because previous attempts to obtain PRCA sera from patients for the development of reference standards have proved largely unsuccessful a panel of nine human monoclonal antibodies (mAbs) against human EPO with defined characteristics was obtained. This panel is intended to be made available to manufacturers and clinical laboratories to allow for the selection and validation of appropriate assays, and for the monitoring of assay performance. The antibody panel is appropriate for use with all ESAs structurally related to human EPO, and contained a range of mAbs with different characteristics – non-neutralizing and neutralizing, and spanning various isotypes and affinities – based on information derived from clinical samples from non-PRCA and PRCA patients. This project was endorsed by the Committee in 2010.

An international collaborative study was conducted to establish the First WHO Reference Panel for antibodies to erythropoietin (human). The panel
was analysed by 18 laboratories from eight countries using their own in-house assay. Results showed that antibody detection varied between laboratories and was dependent on antibody characteristics and the method used. Within- and between-assay repeatability was generally very good with excellent agreement for most laboratories. Accelerated thermal degradation studies demonstrated that panel samples stored at 37 °C for 9 months showed no significant loss of activity. The Committee was informed that 700 vials would be available for use as an international standard.

The Committee considered the report of the study (WHO/BS/2015.2265) and recommended that the proposed panel be established as the First WHO Reference Panel for antibodies to erythropoietin (human).

5.2 Proposed new projects and updates – biotherapeutics other than blood products

5.2.1 Proposed First WHO International Standard for darbepoetin
Darbepoetin is a recombinant erythropoiesis stimulating protein with a longer in vivo half-life compared to recombinant erythropoietin (EPO). A number of darbepoetin BSPs are in development and some have already been licensed. As non-originator products can vary in quality, efficacy and safety there is now a need for a WHO international standard for use by manufacturers and control laboratories in calibrating assays to assess product potency.

A donation of darbepoetin from the originator or from biosimilar manufacturers will be sought for use in a collaborative study. Potency will be assigned in mass units to the darbepoetin standard using an in vitro bioassay.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2273) to develop a First WHO International Standard for darbepoetin and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2018.

5.2.2 Proposed First WHO Reference Panel for in vitro bioassay of erythropoietin (EPO)
The current international standard for EPO, and its recombinant and natural predecessors, were all value-assigned by in vivo bioassay. The EPO unit, used for dosing regimens, therefore remains traceable to an in vivo bioassay, which also remains the pharmacopoeial assay method. Replacement of the in vivo bioassay with an in vitro method is a high-profile objective. However, although a number of in vitro assays for EPO have been developed and described, their adoption as regulatory assays has been hampered by the fact that the two different assay approaches depend on different rate-limiting parameters of the molecule, and show opposite correlations with processes such as sialylation. The validation of in vitro methods is hampered by the absence of system suitability standards
which allow the relationships between structure and function for various assays to be explored.

The proposed panel would span a range of properties, principally pI (a function of the degree of sialylation), to allow users to define the response of in vitro and in vivo bioassay systems to variations in sialylation. Globally, there are numerous EPO manufacturers, pharmacopoeias and control laboratories who would seek access to such materials.

The Committee considered the document (WHO/BS/2015.2273) outlining the establishment of a panel of bioassay reference reagents for EPO but was not convinced of the utility of such a panel in the in vitro bioassay of EPO and did not endorse the proposal.

5.2.3 Proposed First WHO international standards for pegylated-interferon-α-2a and pegylated-interferon-α-2b

Despite the availability of new therapeutic options for HCV infection, which affects 150–180 million people worldwide, the standard therapy for the treatment of chronic hepatitis C in many countries continues to be pegylated-interferon-α (peg-IFN-α) in combination with ribavirin. Peg-IFN-α-2a and peg-IFN-α-2b are licensed in the USA and Europe, and are available globally. WHO added peg-IFN-α to its Model List of Essential Medicines in 2013. Alternative products have been marketed or are in development in several counties, and these products differ in their biological properties both from the originator product and from the parental unmodified IFN-α-2 forms.

Currently there is no international standard for peg-IFN-α products. International standards for unmodified IFN-α forms are however available, and may have been used by some manufacturers to determine the activities of their products. As potency-determination practices are likely to vary among manufacturers, this can result in products with discrepant potencies. Therefore, reference international standards would be very useful for the in vitro assay of product biological activity. Due to the complexities associated with these products it was proposed that several different preparations be evaluated, and assessment made of the feasibility of developing suitable standards for peg-IFN-α-2a and/or peg-IFN-α-2b for use by manufacturers and control laboratories in calibrating assays for the potency evaluation of such products in order to ensure their clinical safety and efficacy. The biological activity of products would be assessed using appropriate bioassays and a collaborative study organized to assign a unitage to the international standards. Donations of peg-IFN-α from the originator product manufacturer and from alternative product manufacturers will be sought.

The Committee considered the proposal (WHO/BS/2015.2273) and expressed reservations concerning both the feasibility of obtaining candidate materials for the proposed study and the relevance of in vitro results that had
not been shown to correlate with current in vivo test results. The Committee recommended that an exploratory study be undertaken to compare unmodified IFN-α with peg-IFN-α products, and that the results be reported to the Committee in due course.

5.2.4 **Proposed First WHO Reference Reagent for TGN1412-like functional activity**

The severe adverse events that occurred during a Phase I clinical trial of the anti-CD28 superagonist TGN1412 highlighted the need for improved in vitro assays to better predict the risk of cytokine release syndrome following administration of monoclonal antibodies to humans. Although a number of cytokine release assays (CRAs) had since been developed these were of widely varying sensitivity. One problem faced by many investigators was the difficulty in sourcing a positive control with TGN1412-like activity. The availability of a TGN1412 analogue would greatly facilitate assay development and application by manufacturers, contract research organizations, and regulatory and research laboratories.

A TGN1412 analogue (NIBSC code NIB28SA-G4) had been produced by NIBSC with subsequent experiments, including a pilot fill, demonstrating no loss of functional activity in a PBMC/HUVEC co-culture CRA. It was proposed that production fill material would now be evaluated in a multicentre collaborative study for its suitability as a positive control for CRAs. Negative control material would also be provided for the collaborative study.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2273) to develop a First WHO Reference Reagent for TGN1412-like functional activity, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2016.

5.2.5 **Proposed First WHO Reference Reagent for Pam₃ CSK₄**

The inadvertent contamination of parenteral products by endotoxin and non-endotoxin pyrogens can potentially lead to life-threatening, uncontrolled inflammatory events. Assays currently used to identify contamination (bacterial endotoxin test and rabbit pyrogen test) have a number of inherent limitations and rely upon animal products and testing. Significant efforts have therefore been made to establish alternative in vitro methods based upon the use of human monocytes. This has resulted in the development of a number of monocyte activation test (MAT) assays of widely varying sensitivity.

Sourcing a reliable positive control for monocyte activation, other than endotoxin, has proved to be problematic. The availability of an alternative toll-like receptor ligand would greatly facilitate assay development and application by manufacturers, contract research organizations, and regulatory and research laboratories.
The toll-like receptor ligand Pam₃CSK₄ reliably activates monocytes when using two of the most widely used MAT assays. It was proposed that, in coordination with EDQM, this material be evaluated in an NIBSC multicentre collaborative study for its suitability as a positive control for MAT assays employed in assessing pyrogenic contamination of parenteral products.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2271) to develop a First WHO Reference Reagent for Pam3 CSK4 for use in MAT assays, made a number of recommendations regarding potential cell sources, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2017.

5.2.6 Proposed stability monitoring of the First WHO International Standard for pegylated granulocyte colony-stimulating factor

The First WHO International Standard for pegylated granulocyte colony-stimulating factor was established by the Committee in 2013 and is the primary standard for in vitro assays of the biological activity of pegylated granulocyte colony-stimulating factor preparations.

The Committee was informed that samples of the current standard stored at elevated temperatures (20 °C, 37 °C and 45 °C) for approximately 2 years had been tested concurrently with samples stored at the recommended temperature of −20 °C and with baseline samples stored at −70 °C in GNFS-60 bioassays. The calculated potencies of the samples relative to the baseline sample were used to fit the Arrhenius model for accelerated thermal degradation, and resulted in a predicted loss in activity of 0.031% per year when stored at −20 °C. Extended studies after 20 years or upon standard replacement (whichever was sooner) were now being proposed in order to confirm the appropriate stability of the current standard.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2260) and looked forward to the subsequent submission of the stability study outcome to the Committee in due course.

All reference materials established at the meeting are listed in Annex 6.

6.1 WHO International Standards and Reference Reagents – blood products and related substances

6.1.1 First WHO Reference Reagent for anti-A and anti-B in serum and plasma for use in haemagglutination assays

Testing for high-titre anti-A and anti-B in serum and plasma using haemagglutination assays is an important step in minimizing the risk of causing clinically significant haemolysis when blood components rich in plasma containing high titre anti-A/B are transfused to patients of blood groups A, B or AB. Such testing can also facilitate the transplanting of mismatched kidneys from living donors which can be performed successfully if the recipient has sufficiently low levels of anti-A and anti-B, and can also identify high-titre anti-A/B plasma for exclusion from the manufacture of blood products.

A lyophilized serum preparation with high anti-A and anti-B titres (NIBSC code 14/300) was evaluated in an international collaborative study for its suitability to serve as a WHO reference reagent for the standardization and control of haemagglutination titrations for anti-A and anti-B in serum and plasma. Two plasma-based reserve preparations – NIBSC codes 14/304 (high-titre anti-A) and 14/208 (high-titre anti-B) – were also included in the study. Twenty three laboratories in 12 countries tested all three preparations, with 21 laboratories using the recommended methodologies of direct agglutination at room temperature (DRT) using DiaMed neutral gel cards, and indirect antiglobulin testing (IAT) using DiaMed anti-IgG and/or LISS/Coombs gel cards. Up to a 64-fold variation was observed in reported titres per preparation. No reactivity with group A red blood cells (RBCs) was reported for 14/208 and none of the preparations reacted with group O RBCs.

Study results indicated that haemagglutination tests can exhibit wide inter-laboratory variation, even when using a common procedure, and demonstrated the need for international reference reagents with “nominal” titres to facilitate inter-laboratory comparisons and allow sample titres to be reported relative to the reference titres. The establishment of 14/300 as a WHO reference reagent for high-titre anti-A and anti-B in serum, with assigned nominal anti-A and anti-B titres, would facilitate the global standardization of haemagglutination testing in patient samples, and will allow for identification of more consistent cut-off titres for various applications such as ABO-incompatible renal transplants.
The Committee considered the report of the study (WHO/BS/2015.2258), made several suggestions on the information to be included in the Instructions for Use (including an indication of the range of assay types used in the study and of the data used for unit assignment), and recommended that candidate material 14/300 be established as the First WHO Reference Reagent for anti-A and anti-B in serum and plasma for use in haemagglutination assays with nominal anti-A and anti-B titres of 128 and 256 assigned for DRT and IAT respectively.

6.1.2 Fifth WHO International Standard for blood coagulation factor IX (concentrate)

Accurate potency labelling against robust and reliable reference standards is of paramount importance in ensuring the efficacy of clinical products based on high-purity blood coagulation factor IX (FIX) and prothrombin complex concentrates used in the treatment of congenital and acquired factor IX deficiency. Due to depletion of the current WHO international standard a collaborative study was undertaken with the following three main aims: (a) to add an FIX antigen value to the Fourth WHO International Standard for blood coagulation factors II, VII, IX X (plasma); (b) to establish a replacement international standard for blood coagulation factor IX (concentrate); and (c) to investigate the suitability of the replacement international standard as a potency standard for purified full-length recombinant FIX.

For the assignment of an FIX antigen value to the Fourth WHO International Standard for blood coagulation factors II, VII, IX, X (plasma), 15 laboratories generated 17 sets of data for analysis. Only five of these data sets exhibited an intra-laboratory geometric coefficient of variation (GCV) greater than 10%. There was also good inter-laboratory agreement with an observed GCV of 7.9%. Based on the data analysis, it was recommended that an FIX antigen value of 0.90 IU/ampoule be added to the label of above international standard.

For the value assignment of the Fifth WHO International Standard for blood coagulation factor (concentrate) relative to the current international standard two plasma-derived candidate materials (NIBSC codes 14/148 and 14/162) were evaluated by 50 laboratories from 18 countries. The laboratories returned a total of 55 sets of clotting assay results and 15 sets of chromogenic assay results for analysis. Intra-laboratory variability was reasonably low with the majority of GCVs below 10%. Lower inter-laboratory agreement was observed for candidate material 14/148 than for candidate material 14/162. Although there were no discrepancies between clotting and chromogenic assays for either sample, a significantly lower potency (approximately –6%) was obtained for candidate material 14/162 with clotting assays when buffer rather than FIX-deficient plasma was used as a pre-diluent. It was recommended that candidate material 14/148 be established as the Fifth WHO International
Standard for blood coagulation factor IX (concentrate) with an assigned potency of 10.5 IU/ampoule.

The study also investigated the comparability of the plasma-derived concentrate standard with full-length recombinant FIX products and assessed the feasibility of establishing an international standard for recombinant FIX. All three currently licensed full-length recombinant FIX products were represented in the evaluation of two recombinant candidate materials, and data received from 49 laboratories. Despite data analysis indicating that a recombinant standard for recombinant products could minimize assay discrepancies for some products and improve inter-laboratory agreement, a number of objections had been raised in relation to potential adverse impacts on the amount of protein in the final products and the use of multiple international standards for the same coagulation factor. Following evaluation by experts nominated by the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) and subsequent discussions with the ISTH/WHO Liaison Group, it had been decided not to request the Committee to consider the establishment of an international standard for recombinant FIX.

Committee discussions centred on the need for an international standard for recombinant FIX and on the inherently complex difficulties of establishing such a standard in light of the different recombinant FIX preparations now in the development pipeline. Despite the currently unresolved scientific and practical issues, further work on the possible establishment of an international standard for recombinant FIX was encouraged given the likely future need to standardize the potency of such products.

The Committee considered the report of the study (WHO/BS/2015.2261) and recommended that candidate material 14/148 be established as the Fifth WHO International Standard for blood coagulation factor IX (concentrate) with an assigned value of 10.5 IU/ampoule. The Committee further recommended that an FIX antigen value of 0.9 IU/ampoule be assigned to the Fourth WHO International Standard for blood coagulation factors II, VII, IX, X (plasma). For both these standards the Committee requested that consideration be given to specifying the post-reconstitution IU/ml value in the Instructions for Use.

6.2 Proposed new projects and updates – blood products and related substances

6.2.1 Proposed First WHO International Standard for activated blood coagulation factor X

Activated blood coagulation factor X (FXa) sourced from bovine plasma and established in 1975 is currently being used as a non-WHO reference material for the standardized measurement of the FXa content of activated prothrombin complex concentrate (PCC) used in the treatment of haemophilia. In addition,
it is also being used in the investigation of factor X (FX) concentrate, an orphan drug for the treatment of FX deficiency, currently in clinical trials. The suitability of bovine FXas as a reference material to meet regulatory requirements in this area is in question with the material also having been filled externally with no stability data or information on the uniformity of the fill available. It was therefore proposed that the material be replaced with a human preparation of FXa.

Potential candidate material for this purpose was identified from three sources including an internal reference material prepared from human plasma donated by the manufacturer of the PCC FEIBA. One candidate material (NIBSC code 15/102) was shown to be active and homogeneous.

In the proposed collaborative study, the activity of 15/102 will be measured relative to the bovine standard (NIBSC code 75/595) and local FXa standards using chromogenic and clotting methods. Potency assignment will be performed relative to 75/595 or an independent arbitrary unit assigned if this is not possible. The proposed uses of the international standard are to measure FXa levels in PCCs and assess FXa content as a contaminant of FX concentrates. Other potential uses include the measurement of FXa inhibitors, the measurement of FXa in heparin assays and the measurement of truncated FXa now in development as a reversal agent.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2274) to develop a First WHO International Standard for activated blood coagulation factor X.

6.2.2 Proposed First WHO Reference Panel for procoagulant activity of human immunoglobulin

In 2012, a proposal had been put to the Committee to endorse the development of matrix-related reference materials to support assay methods used to measure procoagulant activity in human immunoglobulin products. Initial indications were given that the proposed reference materials were not well defined and the decision was made not to proceed with further discussion at that point. At the same time, a reference reagent for FXIa was established, followed in 2013 by the establishment of an international standard for FXIa to support its measurement in immunoglobulin products. In addition, global stakeholders continued to work on the development and refinement of assays for procoagulant activity.

The Committee was informed that the international standard for FXIa is now helping to harmonize FXIa assay methods, and is also used by regulators and manufacturers to develop other assay methods such as the Non-Activated Partial Thromboplastin Time and Thrombin Generation Assay. Because of the matrix heterogeneity of immunoglobulin products, it was clear that in addition to the purified FXIa standard, matrix-related reference preparations will also be required. Such matrix-related preparations may be superior to purified FXIa in
standardizing procoagulant activity assays that are not amenable to estimation in IUs.

Although the measurement of the procoagulant activity of human immunoglobulins remains an unresolved issue, a degree of commonality was now apparent between different strategies, and agreement on further collaboration had been reached. A working group had been convened comprising representatives of the principal organizations and agencies working in this area. A large collaborative study was now being proposed to clarify the need to establish the relevant reference preparations as a WHO reference panel and it was proposed that the working group report back to the Committee in 2016.

Following discussion of the proposal (WHO/BS/2015.2274), the Committee noted the intended collaborative study and looked forward to being updated on its progress in 2016. An indication was also made by the Ministry of Food and Drug Safety, Republic of Korea of its interest in joining the working group process.

6.2.3 Proposed Fifth WHO International Standard for thromboplastin (human, recombinant)

International Standards for thromboplastins are used to: (a) determine the International Sensitivity Index (ISI) for commercial or local prothrombin time (PT) test reagents and instruments, which is needed to determine the international normalized ratio (INR) in patients receiving vitamin K antagonist; (b) calibrate the INR for whole blood coagulation monitors (point-of-care tests); and (c) certify frozen or freeze-dried plasma for local INR determination. In general, any new thromboplastin standard should be calibrated with a similar reference thromboplastin (like-to-like principle). As stocks of the current WHO international standard are low, and will be exhausted within a year, a replacement standard is now required. It was intended that the replacement would be calibrated against the current international standard.

An international collaborative study has therefore been proposed to assign ISI values to candidate replacement preparations and to evaluate their suitability according to predetermined criteria. A candidate for human recombinant thromboplastin had now been prepared (freeze-dried in sealed ampoules) and assigned the provisional NIBSC code 14/001. Corresponding reconstitution fluid had also been prepared in sealed ampoules. The study would involve approximately 20 laboratories. PT determination will be performed by manual tilt tube technique on 10 different days using blood samples from two healthy subjects and six patients per day that have been treated with vitamin K antagonists to yield an INR of 1.5–4.5. Lyophilized control plasma will be tested in parallel. Accelerated degradation studies will be performed. As long-term stability studies (at −20 °C) would not be completed within the time frame of the project future studies should be considered.
Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2274) to develop a Fifth WHO International Standard for thromboplastin (human, recombinant).

6.2.4 Proposed Fifth WHO International Standard for thromboplastin (rabbit, plain)

As outlined above, thromboplastins used in the PT test for the laboratory control of oral anticoagulant treatment must be calibrated against international standards to determine the ISI necessary to convert PT results into an INR. Feedback from users has indicated that the calibration of a given thromboplastin is generally more precise when performed against an international standard of similar composition, and from the same species. For this reason, WHO therefore maintains international standards for different species. As stocks of the current WHO international standard for rabbit thromboplastin are low, and will be exhausted within a year, a replacement standard is now required. It was intended that the replacement would be calibrated against the current international standard.

As part of the international collaborative study on human prothrombin outlined above in section 6.2.3 a rabbit thromboplastin candidate material has also been prepared and assigned the provisional NIBSC code 15/001. Appropriate reconstitution fluid has again been prepared in sealed ampoules.

During discussion, clarification was sought on why the technical specifications between the two standards were not identical with the range of the rabbit preparation being slightly higher. This appeared to be a reflection of the property of the rabbit brain lysate which needed to be dosed slightly higher than the human recombinant standard to yield equivalent INR values in the respective assays.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2274) to develop a Fifth WHO International Standard for thromboplastin (rabbit, plain).
7. International reference materials – In vitro diagnostic device reagents

All reference materials established at the meeting are listed in Annex 6.

7.1 WHO International Standards and Reference Reagents – in vitro diagnostic device reagents

7.1.1 First WHO International Standard for JC virus DNA for NAT-based assays

JC virus (JCV) is a member of the Polyomaviridae family and is typically acquired in childhood, resulting in an estimated worldwide seroprevalence of 50–90% by adulthood. Following primary infection, the virus establishes latency and the vast majority of individuals remain asymptomatic. Under conditions of immunodeficiency and immune suppression JCV can establish lytic infection in oligodendrocytes leading to demyelination resulting in progressive multifocal leukoencephalopathy (PML). PML presents with progressive neurological deficits, including motor and sensory defects, with a mortality rate of 30–50% within the first few months of diagnosis. Although the incidence of PML remains highest in HIV-1 infected individuals (~85% of cases), cases have also recently been detected among relapsing remitting multiple sclerosis patients undergoing monoclonal antibody treatment with Natalizumab, an immunomodulatory therapy.

The molecular detection of JCV DNA in cerebrospinal fluid is frequently used as a confirmatory diagnosis of PML and is also relied upon during post-treatment monitoring. As highlighted in external quality assessment (EQA) programmes, the absence of a standardized reference preparation currently prevents the assured determination of accurate and comparable viral load. It is envisaged that the proposed international standard will be used by clinical diagnostics laboratories and IVD manufacturers for the calibration of secondary reference reagents and working standards.

An international collaborative study was conducted to evaluate a candidate reference material (NIBSC code 14/114) for use in the standardization of JCV NAT-based assays. The freeze-dried whole virus preparation JCV 1A strain, formulated in a universal buffer, was analysed by 23 laboratories in 14 countries using their own routine NAT-based assays for JCV detection. The freeze-dried candidate was tested alongside its liquid equivalent, as well as patient samples and spiked clinical samples. Despite a large degree of inter-laboratory variation in the estimates obtained, overall results demonstrated the suitability of the candidate material 14/114, with analysis of its dilution in different matrices indicating no matrix effects, highlighting its potential utility in the calibration of multiple clinical samples. Furthermore, accelerated thermal degradation studies performed at 4 and 8 months demonstrated the stability of
the candidate material at temperatures used for storage (−20 °C) and laboratory manipulation (4–20 °C), as well as at the 37–45 °C ambient temperatures typically encountered during global shipment. Further real-time stability studies were now in progress to assess the long-term stability of the candidate material. The Committee was informed that 4103 vials would be available for use as an international standard.

Discussion was held on the potential issues relating to the use of a single subtype, the possibility of matrix effects and the associated issue of commutability. The Committee was informed that although the commutability aspects of the study had been limited, there was nothing to indicate that proposed material would not be suitable, and the possibility of a wider commutability study was being discussed with study participants and other stakeholders. Discussion also centred on the assignment of a unit based on quantitative data or on combined qualitative and quantitative data. Despite an observed discrepancy between qualitative and quantitative findings, the Committee concluded that clinical decision-making for JCV relies primarily on determining the presence or absence of virus rather than its quantitation and the use of a combined value would be appropriate.

The Committee considered the report of the study (WHO/BS/2015.2259) and recommended that the candidate material 14/114 be established as the First WHO International Standard for JC virus DNA for NAT-based assays with an assigned potency of 7.0 log_{10} IU/ml.

7.1.2 First WHO International Standard for BK virus DNA for NAT-based assays

BK virus (BKV) is a member of the Polyomaviridae family and is typically acquired in childhood, resulting in a seroprevalence of ~90% worldwide by adulthood. Following primary infection the virus establishes latency and the vast majority of the population remains asymptomatic. Renal transplantation and haematopoietic stem cell transplantation (HSCT) patients undergoing immunosuppression are susceptible to latent BKV reactivation, which can result in BKV-associated nephropathy (BKVAN) characterized by interstitial nephritis and/or urinary tract stenosis. Affecting up to 10% of all renal transplant patients, BKVAN can cause allograft loss in up to 60% of such affected recipients. In HSCT patients, BKV reactivation can present with haemorrhagic cystitis that can be associated with significant morbidity and mortality.

In the absence of an effective antiviral to treat BKV reactivation, the management of renal transplant patients requires rigorous post-transplantation monitoring of BKV viral load using quantitative polymerase chain reaction (PCR) analysis of urine and plasma at specified time points. A BKV viral load
of ≥ 4 log_{10}/ml for > 3 weeks is presumed predictive for BKVAN, upon which a reduction in immunosuppression is recommended. A standardized reference preparation is therefore needed for accurate and comparable viral load determination. It is envisaged that such a reference preparation would be used by clinical diagnostic laboratories and IVD manufacturers for the calibration of secondary reference reagents and working standards.

An international collaborative study was conducted to evaluate candidate materials for use in the standardization of BKV NAT-based assays. Two candidate samples of freeze-dried whole BKV preparations (NIBSC codes 14/202 and 14/212) formulated in a universal buffer were assessed by 33 laboratories from 15 countries, each using their routine NAT-based assays for BKV detection. The freeze-dried candidates were tested alongside their liquid equivalents, two patient samples and a BKV plasmid construct. For both candidate materials a good degree of assay harmonization was already evident amongst laboratories, and expression of the data as a relative potency for both candidate samples suggested that either could serve as a suitable candidate for an international standard. Of the two candidate samples, 14/212 was considered to be the most suitable based on its geographical representation of the most relevant circulating strain in Europe and the United States. Accelerated thermal degradation stability studies performed at 3 months demonstrated that the candidate material was stable, and exhibited no loss of titre at temperatures used for storage (−20 °C), laboratory manipulation (4–20 °C), as well as at the 37–45 °C ambient temperature typically encountered during global shipment. Further real-time stability studies were now in progress to assess the long-term stability of the candidate material. The Committee was informed that 4092 vials would be available for use as an international standard.

Discussion was held on the assignment of the unit with agreement reached that in light of the minority representation of qualitative assays, the under quantification typically seen in such assays and the primary requirement to quantify BKV viral load for the management of renal transplant patients a unit should be derived solely from the quantitative data obtained. Although the choice of subtype for the candidate material was also approved, there was also discussion of the potential value of creating a subtype panel that included subtype 4 as this was also known to be clinically relevant. It was agreed that NIBSC would investigate this need with stakeholders.

The Committee considered the report of the study (WHO/BS/2015.2270), indicated that the Instructions for Use should clearly specify that assigned unitage was based on quantitative NAT assays only, and recommended that the candidate material 14/212 be established as the First WHO International Standard for BK virus DNA for NAT-based assays with an assigned potency of 7.2 log_{10} IU/ml.
7.1.3 Fifth WHO International Standard for hepatitis C virus RNA for NAT-based assays

HCV is a bloodborne virus that causes both acute and chronic liver disease. HCV is spread by contact with infected body fluids, predominately through the practice of needle sharing. It is estimated that up to 150 million people worldwide are infected with HCV with currently no vaccine available. Although HCV infection can be detected using both serological and molecular methods the latter are preferred in many settings due to their ability to detect early infection.

An international standard for HCV RNA for NAT-based assays has been available since 1996 with the current fourth international standard having been established in 2011. However, concerns have been raised over the stability of the current material with users reporting a 0.2 log_{10} decrease in titre when the material is shipped at ambient temperature. In addition, fewer than 50 vials of the current international standard remain. Following a hypothesis that the presence of antibodies provides some stabilizing effects during the lyophilization process, a pilot study was undertaken to assess the stability of HCV products formulated from both antibody positive and negative material. Following assessment by five laboratories using different commercial HCV NAT-based assays, no differences in titre were observed. A full international collaborative study was then conducted, involving 17 laboratories from 11 countries, to assess a candidate replacement material (NIBSC code 14/150) alongside both the current international standard and the previous second international standard. Accelerated degradation data for the candidate material indicated a 0.1 log_{10} loss in titre at 20 °C over 6 months relative to the −20 °C baseline sample. Further real-time stability studies were ongoing.

Following discussion concerning the assignment of a potency value, it was agreed that this would be based upon the quantitative-only data for relative potency assessed against the second international standard. Clarification was also given of the genotype used to prepare the candidate material, and confirmation provided that this had been shown, via sequencing, to be the same genotype used in the previous international standards.

The Committee considered the report of the study (WHO/BS/2015.2262) and recommended that the candidate material 14/150 be established as the Fifth WHO International Standard for hepatitis C virus RNA for NAT-based assays with an assigned value of 5.0 log_{10} IU/vial when reconstituted in 1.1 ml of nuclease-free water.

7.1.4 Fourth WHO International Standard for anti-Toxoplasma gondii (human)

Toxoplasmosis is a disease caused by the parasite Toxoplasma gondii, the congenital transmission of which remains a considerable burden on global health. During pregnancy, serological screening programmes are used in
some settings to determine maternal toxoplasmosis antibody status as part of efforts to prevent infection of the fetus. In addition, toxoplasmosis is also a major cause of mortality among transplant patients. An appropriate antibody standard is therefore required to validate the serological assays used to diagnose toxoplasmosis. In 1994, TOXM was established as the Third WHO International Standard for anti-Toxoplasma serum, human for use by IVD manufacturers, national reference laboratories and hospital laboratories. Since 2000, stocks of TOXM have been low and are now exhausted.

An international collaborative study was conducted involving 16 laboratories from 12 countries to assess the suitability of a candidate material (NIBSC code 13/132) as a replacement international standard for anti-Toxoplasma immunoglobulin, IgA, IgM and IgG. Each ampoule of the candidate material contained 0.5 ml of pooled human plasma taken from six donors who had experienced a recent *T. gondii* infection. The material was compared with TOXM using: (a) agglutination assays for IgA, IgM and IgG; (b) enzyme linked immunosorbent assays (ELISA) and enzyme linked fluorescent assays for immunoglobulin, IgG, IgM and avidity of IgG; (c) immunofluorescence assays for IgG and IgM; and (d) immunoblots for IgM and IgG and the Sabin-Feldman dye tests. For continuity purposes, the first international standard for anti-Toxoplasma IgG (NIBSC code 01/600) was also included in the collaborative study.

Intra- and inter-laboratory variability was generally found to be low with the candidate material 13/132 exhibiting high levels of anti-Toxoplasma IgG and IgM, and a potency intermediate between TOXM and 01/600. The candidate material was also found to be stable at the temperature used for storage (−20 °C) with accelerated thermal degradation studies at 15 months indicating its suitability for long-term use. The Committee was informed that 3695 ampoules would be available for use as an international standard.

Discussion centred on the long-term stability of the IgM based on the data shown, and on issues arising from the less frequent use of the dye test and associated potential for problems in using current assays arising from the reported high IgG titre of the proposed standard. Nevertheless, it was concluded that the proposed standard would be highly useful in the standardization of serological testing for toxoplasmosis and in supporting the appropriate clinical management of this disease.

The Committee considered the report of the study (WHO/BS/2015.2266) and recommended that the candidate material 13/132 be established as the Fourth WHO International Standard for anti-*Toxoplasma gondii* (human) with a unitage of 160 IU/ampoule. An IgG unitage of 263 U/ampoule was assigned and the recommendation made that the high IgM content of the standard be stated in the Instructions for Use. The Committee further recommended that a potential replacement standard be developed with IgG and IgM unitages suitable for use with current analytical methods.
7.1.5 Assignment of a holotranscobalamin value to the First WHO International Standard for vitamin B12 and folate in human serum

Although it is known that vitamin B12 deficiency can lead to anaemia, the accuracy of current methods for measuring its level in the blood has been questioned. Approximately 20% of circulating vitamin B12 is bound to the protein carrier transcobalamin. The resulting complex, holotranscobalamin (holoTC), is the active portion of B12 available to cells and research indicates that its measurement is a more reliable marker of early B12 deficiency than total B12 measurement. In addition, holoTC assays are considered to be more reliable during pregnancy and in patients taking the oral contraceptive pill as the complex is not subject to the physiological drops associated with total B12 levels in these groups.

A first WHO international standard (NIBSC code 03/178) covering both vitamin B12 and folate in human serum had been established in 2005 using pooled human serum. A consensus B12 value of 480 pg/ml had been assigned to this standard, along with a folate value based on liquid chromatography and mass spectrometry methods. The Committee was informed that 2900 ampoules of this material remained. As commercial assays for holoTC were now available, with others likely to be developed shortly, a proposal had been endorsed in 2014 to assign a consensus holoTC value to the current international standard in order to support holoTC measurement approaches.

An international collaborative study involving 12 laboratories in eight countries had been conducted using a variety of in-house and commercial methods to evaluate the holoTC content of the current standard 03/178 and of three patient serum samples. Study results indicated good overall agreement on the holoTC content of 03/178 with good parallelism also observed with the patient samples, demonstrating commutability between the standard and patient materials. Accelerated thermal degradation studies were performed, and indicated an anticipated potency loss of 0.035% per year for samples stored at −20 °C, which was considered to be acceptable.

The Committee noted that the proposed unit for this marker was in pmol/l and not arbitrary WHO IUs. Following discussion it was clarified and accepted that the proposed assignment in pmol/l would lead to greater industry adoption and thus improved assay harmonization. The Committee then considered the report of the study (WHO/BS/2015.2263) and recommended that a holoTC value of 107 pmol/l be assigned to the WHO First International Standard for vitamin B12 and folate in human serum.

7.1.6 First WHO International Standard for human C-peptide

Human C-peptide is a single chain peptide of 31 amino acids that is synthesized in pancreatic beta cells as the connecting peptide for the A and B chains of
insulin in the proinsulin molecule. The measurement of C-peptide has a number of important clinical uses where the monitoring of beta cell function and endogenous insulin levels are required. Human C-peptide concentrations are measured by immunoassay using patient serum, plasma or urine and the first reference reagent used for the standardization of such assays comprised ampoules of recombinant human C-peptide with a content of 10 μg/ampoule. Established in 1986, the stocks of this reference reagent were now exhausted and in 2010 the Committee endorsed a proposal for its replacement.

An international collaborative study involving 24 laboratories in 10 countries was conducted to evaluate the suitability of a candidate material (NIBSC code 13/146) for use as a first international standard. Following confirmation of the C-peptide content of a primary calibrant, the candidate material 13/146 was calibrated accordingly and further characterized. Estimates obtained from all laboratories were found to be in good agreement, and indicated a content of 8.64 μg/ampoule. Study results also indicated that the candidate material exhibited appropriate immunological activity and was sufficiently stable to serve as an international standard. Assessment of the commutability of the candidate material using a small cohort of patient samples also indicated that the candidate material 13/146 was likely to be commutable with patient serum and urine samples as measured by current immunoassays. The Committee was informed that 2492 ampoules would be available for use as an international standard.

Discussion took place on how best to address an apparent discrepancy in the reported strengths of the formerly used reference reagent and the proposed international standard, especially during assay revalidation, and on the acceptability of using SI units instead of IUs.

The Committee considered the report of the study (WHO/BS/2015.2256) and recommended that the candidate material 13/146 be established as the First WHO International Standard for human C-peptide with an assigned value of 8.64 μg/ampoule. A clear indication should also be given in the Instructions for Use of the known discrepancy in potency against the former reference reagent.

7.1.7 First WHO Reference Panel for hepatitis E virus genotypes for NAT-based assays

Hepatitis E virus (HEV) is a major cause of acute hepatitis worldwide. The virus is transmitted mainly by the faecal-oral route and is associated with waterborne epidemics in the developing world. Four main genotypes are identified among the HEV strains capable of human infection with different, albeit partially overlapping, worldwide distribution. Genotype 1 is predominantly found in Africa and South-East Asia. Genotype 2 is found in Africa (overlapping in most areas with genotype 1) and also in Mexico (where genotype 3 is also widespread).
Genotype 3 is also found in Australia, Europe, North America, South America and Northern Asia. Genotype 4 is widespread in South-East Asia (overlapping in different countries with genotype 1) and is also found in Europe (overlapping with genotype 3). HEV belonging to genotypes 3 and 4 has been detected in domestic pigs and in wild animals such as wild boars and deer, with animal contact or the consumption of under-cooked meat being a likely a source of infection in humans. Infection with genotype 1 can lead to severe consequences in pregnant women and in individuals with underlying liver disease. There is also increasing evidence of persistent infection in immunocompromised patients.

The First WHO International Standard for hepatitis E virus for NAT-based assays was established in 2011. In the same year, the Committee also endorsed a proposal to establish a First WHO Reference Panel for hepatitis E virus genotypes for NAT-based assays comprising all four main genotypes, as well as important sub-genotypes.

An international collaborative study involving 24 laboratories from 14 countries was conducted to evaluate a candidate panel comprising eight strains from plasma and three strains from stool, diluted in pooled plasma. Samples were concurrently evaluated against the current WHO international standard using a range of different qualitative and quantitative in-house and commercially available assays. Simultaneously, a biological reference preparation for HEV genotype 3f was calibrated, initiated by the Council of Europe and the European Commission in support of the implementation of HEV NAT-based screening for solvent/detergent-treated plasma in Europe. Prior to the collaborative study, the stability of the lyophilized samples was studied with stool-derived HEV genotypes 1 and 2 found to be unstable upon storage at elevated temperatures. This effect could be prevented by the addition of stabilizers. Data on the potency of panel members and on the candidate biological reference preparation were presented.

During discussion it was suggested that information be included on the stabilization of the stool-derived material and on the nature of the stabilizers used. The inclusion of the collaborative study results for potency ranges was also suggested as this might help inform users of the variability of the results for the included genotypes. Concerns were expressed however that users might attempt to use the reference panel for quantitative purposes for which it was not intended.

The Committee considered the report of the study (WHO/BS/2015.2264) and recommended that the candidate panel be established as the First WHO Reference Panel for hepatitis E virus genotypes for NAT-based assays. Although no unitage would be assigned to the panel members, the geometric means and ranges for each panel member would be provided in the Instructions for Use for
information only, along with information on the stabilizers used for the stool-derived viruses.

### 7.1.8 Extension of the First WHO Repository of platelet transfusion relevant bacterial strains

The bacterial contamination of platelet concentrates remains a persistent problem in transfusion medicine. For method validation and assessment of blood-safety measures, the use of a repository of bacterial strains able to proliferate in blood components under normal storage conditions is crucial. In 2010, the Committee had established the First WHO Repository of platelet transfusion relevant bacterial strains, consisting of four bacterial strains. At the same time a proposal for the future expansion of the repository was endorsed by the Committee.

During an international collaborative study involving 14 laboratories in 10 countries, 11 new bacterial strains were evaluated for inclusion in an expanded repository. A total of 15 bacterial strains (the four established repository strains plus the proposed new candidate strains) were sent to study participants. During evaluation, nine of the 11 new strains exhibited sufficient growth properties with seven growing in all laboratories and two growing in 12 of the 14 laboratories. Although two strains did not grow in a sufficient number of participating laboratories, an alternative strain of one of these (*Morganella morganii*; PEI-A-91) was successfully restored to the proposed panel following successful re-testing in eight laboratories.

Discussion topics included the generation of stability data in relation to the replacement strain, and the potential methodological benefits arising from the different growth kinetics of the candidate strains. It was also suggested that other bacterial strains commonly reported as platelet contaminants might usefully be added to the repository at a later stage.

The Committee considered the report of the study (WHO/BS/2015.2269) and recommended that the First WHO Repository of platelet transfusion relevant bacterial strains be extended by 10 strains.

### 7.1.9 First WHO Reference Reagent for Ebola virus antibodies

On 5 October 2015, WHO declared that the 2014/2015 Ebola virus disease outbreak in West Africa continued to constitute a Public Health Emergency of International Concern. It was recognized that the availability of internationally recognized reference materials for antibodies to the Ebola virus would facilitate the standardization of Ebola antibody assays used in epidemiological studies to measure past or present Ebola infection and in vaccine studies to measure antibodies elicited by vaccination in humans. In the absence of such standards, individual laboratories would apply their own reference standards which would
not be harmonized with other laboratories and methods, thus impairing the reproducibility of results between laboratories.

Recommendations emerging from a Technical Workshop on the Standardization of Serological and PCR assays for the detection of Ebola virus held at NIBSC in 2015 included the urgent development of interim Ebola standards and, in the longer-term, of international standards in accordance with published WHO guidelines for formal establishment the Committee.

In an international collaborative study involving 17 laboratories in five countries nine Ebola virus antibody preparations were evaluated for their suitability as an interim WHO standard for Ebola antibody assays. These nine blinded-study samples included three preparations of purified anti-Ebola IgG derived from transchromosomic bovines that had been pre-bled or vaccinated with experimental vaccines, three solvent-detergent-treated plasma samples obtained from three patients who had recovered from Ebola virus disease, one solvent-detergent-treated Ebola-negative plasma and two non-treated plasma pools obtained from volunteers vaccinated with the monovalent formulation of the chimpanzee adenovirus 3-vectored vaccine. Results were obtained using 27 assays covering the three general methodological categories of neutralization of live Ebola virus, neutralization of Ebola pseudotypes or virus-like particles and enzyme immunoassay.

The Committee recognized the urgent need for such a material and congratulated the groups involved on their greatly expedited efforts. Discussions were held on the reasons for a lack of detectable antibodies in vaccinee material and on the sufficiency of the available data. As the studies were not sufficiently complete to establish any of the candidate materials as an international standard, it was decided that the material should be described as a reference reagent with this designation reflected in the unit terminology used.

The Committee considered the original proposal for the study (WHO/BS/2015.2275) and the presentation of its expedited results and recommended that the American Red Cross Ebola virus convalescent sample (sample code 79) be established as the First WHO Reference Reagent for Ebola virus antibodies with an assigned unitage of 1 U/ml.

7.1.10 First WHO reference reagents for Ebola virus RNA for NAT-based assays

The calibration and performance assessment of Ebola virus RNA NAT-based assays requires reference materials that can be used in parallel with clinical samples to standardize or control for every step of the assay, from extraction through to detection and quantification.

An international collaborative study was conducted involving 13 laboratories from seven countries using a range of in-house and commercially
available qualitative and quantitative Ebola virus RNA NAT-based assays. To ensure the biosafety of the materials to be provided to end users, candidate synthetic Ebola virus RNA interim reference materials were developed for assessment in this study with the longer-term goal being to produce an inactivated Ebola virus validated as non-infectious and formulated for use as a fully established international standard. The synthetic reference materials used in the blinded-panel study were developed using two different approaches. One approach utilized a lentiviral-based system to produce both candidate reference materials and in-run controls, with an influenza-like ribonucleoprotein-based system used to produce additional study materials. The resulting synthetic Ebola virus RNA candidate materials produced were non-replicating, non-infectious and suitable for use during the extraction, amplification and detection steps of NAT-based assays.

Although the commutability of the candidate interim standards for clinical samples was not fully assessed due to biosafety concerns, the use of a variety of assay methods and diluents was considered to have addressed some such aspects. An accelerated thermal degradation study at 1 month indicated that the freeze-dried candidates were sufficiently stable for storage at −20 °C and for shipment at ambient temperatures within temperate climate zones. It was recommended however that any interim reference materials established be packed in ice packs or dry ice when shipping to hotter climates. As with the establishment of the First WHO Reference Reagent for Ebola virus antibodies (see section 7.1.9) it was proposed that the interim materials be established as reference reagents.

The Committee considered the original proposal for the study (WHO/BS/2015.2275) and the presentation of its expedited results and recommended that samples 1003 (Ebola NP-VP35-GP-LVV high for use in assays targeting the np, vp35 and gp genes) and 1012 (Ebola VP40-L-LVV high for use in assays targeting the vp40 and l genes) be established the First WHO reference reagents for Ebola virus RNA for NAT-based assays with assigned unitages of 7.5 log_{10} U/ml and 7.7 log_{10} U/ml respectively when reconstituted in 1 ml of nuclease-free water. Although arbitrary these values represent the consensus estimates obtained across the range of quantitative laboratory assays used in the study. The Committee also encouraged the conducting of follow-up studies to investigate commutability with clinical samples. The Committee further recommended that two lower-titre candidate materials (Ebola NP-VP35-GP-LVV low and Ebola VP40-L-LVV low) be established as in-run controls with assigned potencies of 3.5 log_{10} U/ml and 3.7 log_{10} U/ml respectively when reconstituted in 1 ml of nuclease-free water. The Committee noted that approximately 200 vials of each of the interim reference reagents and approximately 4000 ampoules of each of the in-run controls would be available for distribution.
7.2 Proposed new projects and updates – in vitro diagnostic device reagents

7.2.1 Proposed Fourth WHO International Standard for hepatitis B virus DNA for NAT-based assays

Hepatitis B virus (HBV) is a highly infectious virus transmitted via blood and bodily fluids and is known to cause cirrhosis of the liver and hepatocellular carcinoma. Detection of HBV can be performed by serological or molecular methods. Molecular NAT-based assays are routinely used to manage HBV infections with a number of countries also mandating the NAT-based HBV screening of cells, tissues and organs for transplantation. Blood screening using NAT-based assays is not universally mandated but is widely implemented on a voluntary basis.

The Third WHO International Standard for hepatitis B virus DNA for NAT-based assays was established in 2011 and has been used widely for the calibration of new HBV NAT-based assays and of secondary reference materials. The Committee was informed that current stocks would be sufficient for approximately two years and that the development of a replacement standard would need to commence shortly to maintain an adequate supply.

At the time of preparation of the current international standard the bulk material was freeze-dried in two batches (NIBSC codes 10/264 and 10/266). Both the current standard (10/264) and candidate replacement material (10/266) were evaluated in a collaborative study with mean relative potencies of 5.93 and 5.98 log_{10} IU/ml respectively obtained relative to the second international standard. The Committee was informed that 2533 vials of 10/266 were available and a collaborative study would be conducted to compare the potencies of 10/264 and 10/266. In addition, HBV-positive plasma samples would be included to allow for comparison with clinical samples, and calibration of a secondary reference material for HBV NAT-based assays would also be undertaken.

Following a query regarding the labelling of the secondary reference reagent and the expected remit of the Committee in its establishment it was clarified that although the relevant data would be available to the Committee there would be no requirement for its formal establishment.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a Fourth WHO International Standard for hepatitis B virus DNA for NAT-based assays.

7.2.2 Proposed Fourth WHO International Standard for HIV-1 RNA for NAT-based assays

Human immunodeficiency virus (HIV) causes weakening and ultimately failure of the immune system through infection of lymphocyte cells, predominately CD 4+ helper T cells. The progressive loss of such cells leaves the host susceptible to
opportunistic infections, which if left untreated are life threatening. Although advances in drug therapy have played a major role in slowing the spread of disease, the accurate detection and monitoring of infection remain essential to prevention efforts and are key elements in patient care. As the virus is spread through blood and bodily fluids, NAT-based screening for HIV is also a major component of blood transfusion screening protocols.

The Third WHO International Standard for HIV-1 RNA for NAT-based assays was established in 2011. In order to maintain supplies, this standard will need to be replaced within 2 years. The Committee was informed that high-titre stocks of the source material used for the current international standard were still available for use as a replacement standard. Given that the material will be heat-inactivated, non-infectious and derived from ample tissue culture stocks, it would be feasible to produce a batch of 5000–6000 vials. It was intended that a number of HIV-positive plasma donations would also be included in the proposed collaborative study.

Discussion revolved around the most appropriate subtype to be selected for the replacement material. Although previous international standards had been formulated from a stock of subtype B material, it was known that subtype C was now the most prevalent HIV subtype globally. Nevertheless, it was agreed that to maintain continuity with the current international standard that the replacement material would consist of the same subtype B strain. It was also agreed that, availability permitting, the previous first and second international standards should also be included in the collaborative study assessment.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a Fourth WHO International Standard for HIV-1 RNA for NAT-based assays.

7.2.3 Proposed First WHO International Standard for varicella zoster virus DNA for NAT-based assays

Varicella zoster virus (VZV) is a member of the Herpesviridae family and is transmitted via the respiratory route. It is known to cause a two-stage infection with primary infection resulting in chickenpox (varicella) and subsequent virus reactivation resulting in shingles (herpes zoster). Following primary infection, often during childhood, lifelong latency is established in the neurons of the dorsal root ganglia with reactivation often occurring in later in life. As serious neurological complications can arise from untreated infections, accurate determination of assay sensitivity is essential for ensuring correct patient management. Although vaccines are available for both varicella and herpes zoster the degree of implementation varies between countries.

Molecular detection methods are considered the primary diagnostic tool with EQA programmes demonstrating considerable variation in assay sensitivity.
In addition, a number of tests used by diagnostic laboratories are in-house assays. It was therefore proposed that an international collaborative study be conducted to evaluate candidate material for use as a First WHO International Standard for varicella zoster virus DNA for NAT-based assays. A range of in-house and, where possible, commercially available assays would be used to test a characterized cell culture virus supernatant diluted in negative cerebrospinal fluid, along with clinical cerebrospinal fluid samples and/or cultured virus from skin lesions.

Following discussion and further consideration, the Committee agreed that there was a need for standardization in this area endorsed the proposal (WHO/BS/2015.2275) to develop a First WHO International Standard for varicella zoster virus DNA for NAT-based assays.

### 7.2.4 Proposed First WHO International Standard for *Trypanosoma cruzi* DNA for NAT-based assays

*Trypanosoma cruzi* is a protozoan flagellate and is the causative agent of Chagas disease, the elimination of which is the focus of a WHO programme. In addition, the World Health Assembly passed a resolution regarding the need for *T. cruzi* diagnostics. *T. cruzi* is transmitted by bites from Triatominine insects but can also be transmitted via congenital, bloodborne, tissue-borne and oral routes. An estimated 6–7 million people are infected worldwide, mostly in the Latin American region, but also, as a result of global travel, in Europe, Japan, North America and the Western Pacific region.

As no higher-order standard exists for the standardization of *T. cruzi* diagnostic assays it was proposed that an international collaborative study be conducted to evaluate candidate material for use as a First WHO International Standard for *Trypanosoma cruzi* DNA for NAT-based assays.

The Committee acknowledged the need for a standard in this area and requested clarification of the choice of subtype VI material as the main candidate. As the proposed study also included the development of a panel of materials of different subtypes it was felt that its outcome may ultimately determine the correct subtype to be established. In the absence of the immediate resolution of this issue the Committee requested that full clarification of this point be provided at its next meeting. Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a First WHO International Standard for *Trypanosoma cruzi* DNA for NAT-based assays.

### 7.2.5 Proposed First WHO International Standard for *Plasmodium vivax* DNA for NAT-based assays

*Plasmodium vivax* is a protozoan parasite and one of the main causative agents of malaria with up to 300 million cases of infection annually attributable to this parasite. *P. vivax* now causes as many cases of malaria as *P. falciparum* and in
some regions is the most common cause of malaria. Even in low concentrations *P. vivax* is capable of causing severe infection and even death, mostly due to splenomegaly. Addressing *P. vivax* infection is a recognized priority of the WHO Malaria Programme, and it is envisaged that the development of a standard in this area will benefit clinicians, commercial kit manufacturers and researchers.

It was proposed that an international collaborative study be conducted to evaluate candidate material for use as a First WHO International Standard for *Plasmodium vivax* DNA for NAT-based assays. In addition to the candidate material, the study would also assess clinical samples for commutability purposes.

The Committee recognized the need for this standard while noting that the study proposal contained only limited information on a number of key aspects. The Committee suggested that the study should include laboratories in countries where *P. vivax* was endemic, and requested that additional study details be provided at its next meeting. Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a First WHO International Standard for *Plasmodium vivax* DNA for NAT-based assays.

7.2.6 **Proposed First WHO International Standard for *Plasmodium falciparum* antigens**

Globally, an estimated 3.2 billion people in 97 countries and territories are at risk of developing malaria with 1.2 billion of these at high risk (greater than a 1 in 1000 chance of developing malaria in a year). According to latest estimates, 198 million malaria cases occurred globally in 2013 (uncertainty range 124–283 million) resulting in 584 000 deaths (uncertainty range 367 000–755 000).

Rapid diagnostic tests (RDTs) are important tools for surveillance and for malaria case management with approximately 205 million used globally in 2012. The main targets of such products are histidine-rich protein 2 (HRP2), lactate dehydrogenase and aldolase. Although there are many RDT devices currently available detecting a variety of antigens, their validation and quality control are limited as there is currently no international standard for the above antigens. Current standards used for assessing malaria RDTs are based around cryopreserved *P. falciparum* parasites, patient-derived *P. falciparum* (wild-type), *P. vivax* infected blood and a parasite-negative blood panel, housed at the United States Centers for Disease Control and Prevention, and comprising the WHO Malaria Specimen Bank. The collection, preparation, characterization and transport of patient samples for specimen banks are cumbersome and expensive activities difficult to sustain in the long term.

It is proposed that an international standard be developed for use not only in the quality control and standardization of RDTs worldwide but also in monitoring the development of more sensitive diagnostic tests. The proposed
standard would consist of a lyophilized lysate of a *P. falciparum* culture containing sufficient material for the detection of the three main antigens used in malaria RDTs. The candidate material would be assessed in an international collaborative study using capture ELISA and compared against clinical isolates of *P. falciparum* from Africa, Asia and South America as well as a recombinant HRP2 protein.

The Committee agreed that there was a need for this material and recognized that, although not ideal, the use of recombinant HRP2 to represent an antigen that naturally displays variation would be an acceptable compromise. Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a First WHO International Standard for *Plasmodium falciparum* antigens.

### 7.2.7 Proposed First WHO Reference Panel for KRAS codon 12 and 13 mutations

Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations occur in approximately 40% of metastatic colorectal cancers and are also frequently found in lung and pancreatic cancers. About 98% of KRAS mutations are localized in codons 12 and 13. Patients with KRAS mutations do not respond to anti-Epidermal Growth Factor Receptor antibody therapies, and KRAS mutation testing is therefore of clinical importance with > 1 million colorectal cancer diagnoses made globally each year.

During a proposed international collaborative study eight genomic DNAs (one wild-type lymphoblastoid and seven heterozygous tumour cell lines) will be produced and provided as freeze-dried DNA to more than 20 laboratories for characterization using a broad range of Sanger, next-generation and other sequencing technologies. Consideration was also given to the use of a novel standard format used in CE-marked IVDs (stabilized by anhydrobiosis in the commercially available GenTegra inorganic matrix and stored in screw-capped plastic tubes containing 1 µg of material for storage at 20 °C). In any case, the material would take the form of a reference panel since neither digital PCR nor sequencing will yield results measurable in IS units.

Discussion centred on potential matrix effects in the different assays intended to be used in this study arising from the novel formulation. However, no assays performed to date had exhibited such effects. It was suggested the KRAS content should be defined as a percentage of the wild-type gene and the product diluted in wild-type genomic DNA, bearing in mind the potential aneuploidy of the originator cell lines. Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a First WHO Reference Panel for KRAS codon 12 and 13 mutations.
7.2.8 Proposed Fourth WHO International Standard for prolactin (human)

The measurement of serum prolactin by immunoassay is used to evaluate pituitary gland function, to diagnose and monitor prolactinomas and to determine the cause of galactorrhoea, headache, visual disturbance and infertility. The majority of immunoassays are calibrated with respect to the current Third WHO International Standard for prolactin, human (NIBSC code 84/500), stocks of which are now low. The development of a replacement international standard would require the sourcing of human pituitary prolactin, which is not readily available. Potential sources include donors who have previously provided pituitary-derived materials to NIBSC or materials obtained from commercial organizations.

In the absence of such a bulk donation, it was proposed that an international collaborative study be conducted of a batch of ampoules (2000 remaining) that was filled using pituitary prolactin donated by a Swedish manufacturer in the 1980s. Evaluation of this material (NIBSC code 83/573) had been undertaken during the establishment of the current international standard in 1986, using bioassays (in vivo and cell-based), immunoassays and radioreceptor assays. The unitage assigned to 83/573 in that study was 63 IU/ampoule. The same material had also been included as a pituitary preparation in a study held in 2000 to establish the First WHO Reference Reagent for prolactin, human, recombinant. No viral markers have been detected in 83/573 and its dose-response has been demonstrated to be parallel to 84/500. Study participants would include immunoassay manufacturers, experts in the measurement of prolactin and control laboratories, who will be asked to evaluate the current and candidate international standards (84/500 and 83/573), the First WHO Reference Reagent for prolactin, human, recombinant and up to 16 human serum samples containing prolactin within and above the normal range. Accelerated degradation samples (9 months) were available and would be distributed to participants as capacity allowed or measured at NIBSC.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a Fourth WHO International Standard for prolactin (human).

7.2.9 Proposed First WHO Reference Panel for hepatitis E virus antibodies

Diagnosis of infection with hepatitis E virus (HEV) requires a variety of tests including the detection of IgM and (rising) IgG antibodies. The analysis of IgM antibodies in particular is useful for the confirmation of acute infection. Anti-HEV IgG may also be detectable during acute infection. Anti-HEV IgG is also a marker of past HEV infection and is thus important in seroprevalence studies of previous HEV exposure in different populations.
In 1997, a WHO reference reagent (NIBSC code 95/584) originating from a single donor and of unknown genotype had been established by the Committee. Since then there has been growing recognition of the urgent need for an international validation exercise of all existing HEV serology tests based on the known lack of sensitivity and/or specificity of many serological assays. In some cases there appears to have been a significant underestimation of HEV seroprevalence in blood donors and, particularly in the case of IgM assays, significant discrepancies in sensitivity and specificity, as well as cross-reactivity with antibodies to other pathogens.

A project was now being proposed to establish an anti-HEV reference panel which should ideally represent a wide geographical area. Such a reference panel would also allow for the re-evaluation of the current WHO reference reagent if current stock levels justified this, or for the identifying of possible replacements if not.

Following discussion and further consideration, including the need to include genotype information for any proposed panel, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a First WHO Reference Panel for hepatitis E virus antibodies.

7.2.10 Proposed First WHO Reference Panel for HIV-1 p24 antigen

The early detection and monitoring of infection with HIV is a key element in treatment and prevention activities. Although the use of molecular methods allows for early viral detection their cost is often prohibitive in low-income countries. In addition, the genetic diversity of HIV presents a challenge to all assay development with the accurate detection of HIV across all subtypes known to be problematic.

An HIV-1 p24 antigen reference reagent (NIBSC code 90/636) was first established by WHO in 1992 using subtype B patient-derived material that had been inactivated using a solvent detergent treatment. Further development in this area slowed following the emergence of molecular diagnostic techniques in the late 1990s.

Following collaboration between NIBSC and the University of Zurich a panel of samples was developed for use in the assessment of p24 antigen assays. The panel consists of virus-like particle (VLP) constructs generated in tissue culture from eukaryotic expression vectors. The VLPs contain subtype-specific structural Gag proteins cloned from patient RNA extracts. The use of enzymatic proteins (with the exception of PR) from same viral backbone (subtype B) allows for homogenous quantification and input standardization via reverse transcriptase activity.

Initial stability studies at NIBSC on lyophilized materials have demonstrated the potential for the successful production of stable freeze-dried material. The proposed panel would consist of lyophilized VLPs of the HIV-1
subtypes A, B, C, D, F1, G, H, circulating recombinant forms (CRF01_AE, 02_AG, 12_BF, 20_BG) and group O (plus optional HIV-2). Each VLP would be diluted in human plasma to physiologically relevant concentrations that could be traced to the First WHO Reference Reagent for HIV-1 p24 antigen.

The Committee agreed that this was an important project that addressed a current gap in the standardization of HIV detection. Although of global importance there may be greater demand in countries with limited resources. It was therefore suggested that assays (including antigen-only and combination) that are most commonly used in such settings should be used in the proposed study, along with the current WHO reference reagent. The participation of laboratories in the developing world should also be ensured. Committee members requested that both HIV antibody positive and negative clinical samples be included in the study. Additionally there should also be broad and relevant representation of mosaic viruses, parallel testing with seroconversion panels and inclusion of an HIV-2 VLP.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a First WHO Reference Panel for HIV-1 p24 antigen.

7.2.11 Proposed First WHO Reference Panel for Babesia microti antibodies

Babesiosis is a tick-borne zoonosis caused by infection with intra-erythrocytic protozoans of several species of the genus Babesia. Although several Babesia species are transmitted in the United States, B. microti is the most prevalent. Endemic B. microti transmission has also been reported in Canada, parts of Europe and Japan (B. microti-like). The prevalence of such infections in the United States and other countries is poorly understood with the full extent of global transmission believed to be underestimated.

In 2014, a total of 1759 babesiosis cases in 21 states were reported to the national surveillance programme in the United States highlighting the risk of infection outside the nine known endemic states. In addition, areas of endemic transmission are reported to be expanding, particularly in states adjoining the endemic states. Although there is no FDA-licensed test for diagnostic or donor-screening purposes, some donor testing using investigational tests has been conducted in the United States.

It was proposed that CBER develop a First WHO Reference Reagent panel for Babesia microti antibodies. Panel members would be formulated from defibrinated plasma samples taken from humans who had a current or recent B. microti infection. In addition, RBCs from DBA/2 mice infected with B. microti would be used as a source of antigen in immunofluorescence assays. The reference panel would consist of four serial dilutions of the pooled B. microti antibody positive samples and one pooled B. microti antibody negative sample.
The need for this panel was acknowledged by the Committee with discussions held in relation to the degree of assay cross-reactivity between *B. microti* and other species of *Babesia*. Although not specifically tested for in the material proposed for this panel, reports in the literature suggested that some degree of cross-reactivity existed between species. Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to develop a First WHO Reference Panel for *Babesia microti* antibodies.

**7.2.12 Proposed First WHO Repository of red blood cell transfusion relevant bacterial strains**

The initial establishment in 2010 of the First WHO Repository of platelet transfusion relevant bacterial strains and its extension in 2015 (see section 7.1.8 above) represent the first steps in a strategy to establish repositories of transfusion-relevant bacterial strains for all blood components. It was now proposed that a First WHO Repository of red blood cell transfusion relevant bacterial strains also be established for the validation of both bacterial-screening methods and pathogen-reduction systems.

Microbial infection of RBC products and resulting infection in recipient patients is frequently reported, often with fatal outcome. Based on the reported literature and available haemovigilance data, a number of bacterial strains had now been chosen for pre-testing for potential inclusion in the proposed repository based upon their growth properties at 4–6°C for up to 49 days. While some strains showed no growth and were not detectable after 49 days, others were still detectable (and would thus survive in RBC preparations) and some even grew to high titres in less than 14 days. Bacterial reference strains with relevance in RBC transfusion include both Gram-positive bacteria (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* and *Streptococcus pyogenes*) and Gram-negative bacteria (*Klebsiella oxytoca*, *K. pneumonia*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Yersinia enterocolitica*, *Serratia marcescens*, *S. liquefaciens* and *Listeria monocytogenes*). Since growth ability may vary by strain an international collaborative study was proposed. Although spiking experiments to demonstrate bacterial inactivation are performed by manufacturers, the availability of a bacterial reference panel would allow standardized comparisons to be made of different technologies. It was also agreed that the panel should include additional strains that grow reliably in RBCs and which are relevant to transfusion-induced sepsis.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2275) to establish a First WHO Repository of red blood cell transfusion relevant bacterial strains.
8. International reference materials – vaccines and related substances

All reference materials established at the meeting are listed in Annex 6.

8.1 WHO International Standards and Reference Reagents – vaccines and related substances

8.1.1 First WHO International Standard for anti-EV71 serum (human)

Vaccines for the effective prevention of hand, foot and mouth disease caused by enterovirus 71 (EV71) are under development across South-East Asia. The quantification of anti-EV71 neutralizing antibodies in human sera is an important marker and diagnostic tool in assessing the immune status of individuals, and is invaluable in evaluating the immunogenicity of EV71 vaccines.

A proposal to establish a First WHO International Standard for anti-EV71 serum (human) was endorsed by the Committee in 2012. Candidate materials were identified among 62 plasma samples provided by the National Institutes for Food and Drug Control, China. EV71 strains representative of a wide range of B and C genotypes were used for the initial characterization of the candidate serum samples. An international collaborative study involving 17 laboratories from 6 countries was then conducted to evaluate the suitability of two candidate materials (NIBSC codes 14/138 and 14/140) using a wide range of in-house virus neutralization assays. Both candidate materials exhibited good levels of neutralizing antibodies against a wide range of EV71 strains of various genotypes. Furthermore, inter-laboratory variations in neutralization titres were significantly reduced when values were expressed relative to either of the two candidate sera. Stability studies demonstrated that both candidate materials were also stable at temperatures used for storage (−20 °C) and laboratory manipulation (4–20 °C). Accelerated thermal degradation studies at 3 months further indicated that both candidates would be stable for long-term use. Real-time stability studies of samples maintained for 12 months at different temperatures also showed no significant loss of activity in samples held at 20 °C and below relative to those stored −20 °C. It was concluded that both candidate materials would be suitable for use as an international standard, and that in addition a low-titre material (NIBSC code 13/238) could usefully serve as a WHO reference reagent for the standardization of EV71 neutralization assays.

The Committee considered the report of the study (WHO/BS/2015.2267) and recommended that candidate material 14/140 (5000 ampoules) be established as the First WHO International Standard for anti-EV71 serum (human) with an assigned value of 1000 IU/ampoule. Candidate material 14/138 (5000 ampoules) was recommended as a potential replacement international standard in the future with an assigned value of 1090 IU/ampoule. In addition,
material 13/238 was established as the First WHO Reference Reagent for EV71 neutralization assays with an assigned value of 300 IU/ampoule.

8.1.2 First WHO international standards for meningococcal serogroups A and X polysaccharide

For over a century meningococcal disease caused by meningococcal serogroup A (MenA) has caused a significant burden to the region of Africa stretching between Senegal and Ethiopia. In 2010, a conjugate vaccine developed specifically for that area was introduced by the Meningitis Vaccine Project. As a result, the incidence of disease caused by MenA has declined dramatically. However, MenX, a previously rare causative agent of disease, has emerged as a cause of outbreaks in the region. As a result, initiatives are now under way to manufacture a conjugate vaccine similar to those used for MenA, C, W and Y to control disease caused by MenX.

Plain polysaccharide (PS) and conjugate vaccines are primarily evaluated by physicochemical methods to ensure that batches are consistently manufactured. As different assays are employed to quantify the PS content as the potency test of final formulations and bulk intermediates, there is a need for international standards for both MenA and MenX PS to support calibration of the internal references used in different laboratories. In 2011, the First WHO International Standard for meningococcal serogroup C polysaccharide was established for use in quantification assays for polysaccharide MenC-based vaccines. MenA and MenX international standards are designed to be utilized in a similar way, and their proposed development had been endorsed by the Committee in 2013 and 2014 respectively.

An international collaborative study involving 11 laboratories from nine countries, each using their own in-house assay, was conducted to establish a First WHO international standard for meningococcal serogroups A and X polysaccharide. One candidate material for each serogroup was provided for analysis by each of two different manufacturers. Intra- and inter-laboratory repeatability was generally very good with excellent agreement observed between laboratories when the same methods were used for determining the potency of the candidate materials. Both candidate materials (NIBSC codes 13/246 and 14/156) were found to be suitable for use as international standards. Stability studies also demonstrated that the candidate materials were stable at temperatures used for storage (−20 °C) and laboratory manipulation (4 °C). During accelerated thermal degradation studies, a significant reduction in lyophilized plug volume was observed for both candidate materials following storage at 37 °C and 56 °C for up to 1 year for MenA (13/246) and 6 months for MenX (14/156). The amount of PS in the ampoules remained constant under all conditions. Real-time stability, reconstituted and accelerated thermal degradation studies are ongoing.
The Committee considered the report of the studies (WHO/BS/2015.2255 Add 1) and recommended that candidate material 13/246 (3000 ampoules) be established as the First WHO International Standard for meningococcal serogroup A polysaccharide with an assigned value of 0.845 ± 0.043 mg/ampoule; and that candidate material 14/156 (900 ampoules) be established as the First WHO International Standard for meningococcal serogroup X polysaccharide with an assigned value of 0.776 ± 0.089 mg/ampoule.

8.1.3 Third WHO International Standard for diphtheria toxoid for use in flocculation test

Diphtheria is caused by exotoxin-producing strains of the bacterium Corynebacterium diphtheriae. Active immunization against diphtheria is based on the use of diphtheria toxoid (DTxd, a chemically detoxified preparation of diphtheria toxin) to induce protective antibody responses. Diphtheria vaccines form an essential component of the primary immunization schedule of children and have been part of the WHO Expanded Programme on Immunization since its inception in 1974.

DTxd is produced by growing the toxin-producing C. diphtheriae in liquid media and converting the toxin to inactive toxoid by treatment with formaldehyde. The antigenic strength and purity of the bulk toxoid is evaluated by measurement of “limit of flocculation” (Lf) units. Measurement of antigen content in Lf also serves as a good indicator of the consistency of production, and testing of the crude toxin prior to inactivation is recommended for monitoring purposes. Flocculation remains the primary method used by vaccine manufacturers to evaluate toxin and toxoid concentrations in Lf units.

The Second WHO International Standard for diphtheria toxoid for use in flocculation test was established in 2007 and defines the Lf unit. In light of limited remaining stocks of this international standard a project was initiated to calibrate and establish a replacement material. An international collaborative study involving 25 laboratories in 15 countries was conducted to establish a Third WHO International Standard for diphtheria toxoid for use in flocculation test. Candidate material for the replacement standard was provided by one manufacturer for formulation and filling prior to freeze-drying (Preparation A). A similar material from another manufacturer was also provided, and prepared in the same way, for a proposed new Pharmacopoeial Reference Standard for diphtheria toxoid (Preparation B). The study was initiated with the primary aim of calibrating these materials in Lf units using the Ramon flocculation test standardized against the current international standard. Preparation A was found to have a unitage of 1874 Lf/ampoule while Preparation B was found to have a unitage of 714 Lf/ampoule. Intra- and inter-laboratory repeatability was generally good for both preparations. A secondary aim of the collaborative study was to assess the suitability of alternative antigen-detection methods.
for measuring Lf. Comparable results to the original Ramon flocculation test were obtained when using a laser light-scattering platelet aggregometer as the detection system for flocculation. Similarly, Lf results returned by ELISA methods were not significantly different to flocculation, suggesting that ELISA may be used as a suitable alternative to the flocculation test for measuring the Lf of diphtheria toxoid samples, subject to validation.

Real-time stability studies were ongoing and had so far demonstrated that the candidate material (NIBSC code 13/212) was stable at the normal storage temperature (−20 °C) and suitable for use up to 3 months after reconstitution when stored at 4 °C. Accelerated thermal degradation study results suggested that the material would also exhibit suitable long-term stability with a predicted degradation rate of 0.018% per year when stored at −20 °C.

The Committee considered the report of the study (WHO/BS/2015.2254) and recommended that Preparation A (NIBSC code 13/212; 4800 ampoules) be established as the Third WHO International Standard for diphtheria toxoid for use in flocculation test with an assigned value of 1870 Lf/ampoule.

8.2 Proposed new projects and updates – vaccines and related substances

8.2.1 Proposed First WHO international standards for meningococcal serogroups W and Y polysaccharide

Meningococcal disease is found worldwide with severe outbreaks of disease occasionally occurring. Tetravalent conjugate vaccines to prevent disease caused by Neisseria meningitidis serogroups ACWY are used globally. Three ACWY conjugate vaccines are licensed and currently in use. Other conjugate vaccines with different valencies, containing W and Y polysaccharide are in development. Quantification of the total and free (unconjugated) saccharide in conjugate vaccines is of key importance as an indicator of vaccine potency. With the increased use of multivalent conjugates there is a need to accurately quantify each different PS in order to achieve the correct balance of each in the final formulation.

The First WHO International Standard for meningococcal serogroup C polysaccharide was established in 2011, with WHO international standards for MenA PS and MenX PS being established in 2015 (see section 8.1.2 above). The proposed WHO international standards for MenW and MenY PS will allow for the accurate quantification of PS in both the intermediate PS and bulk conjugate components and final vaccine, and will complete the set of international standards used for the quantification of PS in meningococcal conjugate vaccines.

The candidate materials to be used in a proposed international collaborative study would be manufacturer-donated purified PS of vaccine quality with low lipopolysaccharide and protein content. Approximately 10 laboratories
would be involved in evaluating the suitability of the candidate materials for determining MenW and MenY PS content in conjugate vaccine samples. In a departure from previous approaches, it was proposed to use quantitative nuclear magnetic resonance (NMR) results from a single laboratory to assign unitages. The proposed international standards would potentially be suitable for use in three quantitative assays – NMR, high-performance anion-exchange chromatography with pulsed amperometric detection and resorcinol assays.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO International Standard for meningococcal serogroup W polysaccharide, and a First WHO International Standard for meningococcal serogroup Y polysaccharide, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2018.

8.2.2 Proposed First WHO international standards for Vi polysaccharide

Typhoid is a major cause of illness and death among children in developing countries. In 2008, WHO estimated that typhoid fever causes 216,000–600,000 deaths annually. Most of these deaths occur in developing countries, primarily in Asia. In such countries, large segments of the population do not have access to safe water supplies or basic sanitation services, and there are only limited programmes for detecting carriers and preventing them from handling food. The vaccination of local populations has now become economically feasible as vaccine manufacturers in developing countries start to produce Vi PS conjugate vaccines.

There are minimum requirements for the amount of Vi PS per dose of plain or conjugate vaccine. The relative ratio of free PS against total PS, the extent of O-acetylation and molecular size/mass distribution have all been used as important markers of immunogenicity. As the amount of Vi PS in relation to the carrier protein is important for assessing vaccine potency an international standard for Vi PS is required.

Manufacturer-donated GMP-grade Vi PS obtained from *Citrobacter freundii* was found to be immunogenic in a Phase I study and immunologically identical to *Salmonella typhi* Vi PS. In addition a second GMP-grade Vi PS preparation purified from *S. enterica* subspecies Typhi was also being evaluated as a potential standard.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO International Standard for *Citrobacter freundii* Vi polysaccharide and a First WHO International Standard for *Salmonella typhi* Vi polysaccharide, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee.
8.2.3 Proposed First WHO International Standard for anti-Vi polysaccharide serum (human)

The Committee was reminded that following the submission in 2014 of the results of an earlier international collaborative study on the development of an international standard in this area, it had been concluded that further work was required to determine the cause of the significant inter-laboratory and inter-assay variation observed. In response to that request, it was determined that the potential causes of such variation included the technical expertise of laboratories, poor binding of Vi PS to ELISA plates and the quality of IgG used in the reference reagents.

A revised collaborative study was therefore being proposed in which the original candidate material would be analysed by five of the original participant laboratories. The candidate material would be analysed alongside nine freeze-dried preparations of anti-Vi IgG positive sera obtained from volunteers in a Phase I clinical trial of Vi PS-tetanus conjugate. Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to continue to develop a First WHO International Standard for anti-Vi polysaccharide serum (human), and agreed that the revised collaborative study should proceed after further discussions with appropriate experts. The results of this study were expected to be submitted to the Committee in 2017.

8.2.4 Proposed First WHO Reference Panel for non-typhoidal salmonella O-antigen polysaccharides

Invasive non-typhoidal salmonella disease is caused by Salmonella enterica serovars Typhimurium (O4,5 LPS) and Enteritidis (O9 LPS), and is a leading cause of bacteraemia in sub-Saharan Africa, particularly in infants and immunocompromised individuals. The disease has a high case fatality rate and multidrug resistance hampers effective treatment. Field studies have shown that increased serum levels of anti-O IgG in young children reduce the chances of reinfection with S. enterica serovar Typhimurium. Anti-O IgGs are also bactericidal in vitro.

Vaccine manufacturers in developing countries have started to produce bivalent vaccines containing O4,5 and O9 antigens representing these serovars. As several vaccines targeting the O-antigen PS will be entering clinical development, a common global standard is now required for the quantification and testing of quality parameters of O4,5 and O9 PS used in bioassays to measure the potency of these vaccines and to compare the immunogenicity of various vaccines. At present, three types of bivalent vaccines (conjugate, attenuated oral and outer membrane) are in development, and it was therefore proposed that an international collaborative study be undertaken to establish a First WHO
Reference Panel for non-typhoidal salmonella O-antigen polysaccharides. The candidate materials would be of GMP standard.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO Reference Panel for non-typhoidal salmonella O-antigen polysaccharides, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2019.

8.2.5 Proposed First WHO international standards for antibodies (human) to *Salmonella enterica* serovars Typhimurium (O4,5 LPS) and Enteritidis (O9 LPS)

As outlined above in section 8.2.4 invasive non-typhoidal salmonella disease is caused by *Salmonella enterica* serovars Typhimurium (O4,5 LPS) and Enteritidis (O9 LPS) and vaccine manufacturers in developing countries have started producing bivalent vaccines containing O4,5 and O9 antigens. At present, in-house or commercially available antisera and immunoassays are used to characterize vaccine immunoreactivity. Given the potential importance of such vaccines in controlling disease there is now a need to develop international standards for anti-O4,5 serum (human) and anti-O9 serum (human). It was proposed that an international collaborative study be undertaken to establish international standards in this area. It was intended that several vaccine manufacturers would be approached as potential sources of clinical samples. Sera from volunteers taking part in Phase I clinical trials would also be collected and the pooled material screened for IgG reactive with O4,5 or O9 antigen.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO International Standard for antibodies (human) to *Salmonella enterica* serovar Typhimurium (O4,5 LPS) and a First WHO International Standard for antibodies (human) to *Salmonella enterica* serovar Enteritidis (O9 LPS), and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2019.

8.2.6 Proposed First WHO International Standard for *Salmonella enterica* serovar Paratyphi A O2 polysaccharide

Enteric fever is endemic in South and South-East Asia and is co-localized with typhoid. In 2008, WHO estimated that > 27 million cases of enteric fever occurred annually, and case fatality rates of up to 5% have been reported, mostly in children < 5 years of age. The incidence of Paratyphi A varies by region but in general ranges from 15–25% of all enteric fever cases. In a small number of areas incidences of > 50% have been reported.
Several developing country vaccine manufacturers are producing O2 PS specific for paratyphoid to be combined with typhoid vaccines. These vaccines are expected to enter clinical trials within two years. A common global standard is thus required for the quantification and testing of quality parameters of O2 PS used in bioassays to measure the potency of these vaccines and to compare the immunogenicity of various vaccine formulations. It was proposed that an international collaborative study be undertaken to establish the First WHO International Standard for *Salmonella enterica* serovar Paratyphi A O2 polysaccharide. Candidate materials would be of GMP standard and suitable for use in both immunoassays and physicochemical assays.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO International Standard for *Salmonella enterica* serovar Paratyphi A O2 polysaccharide, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2018.

**8.2.7 Proposed First WHO International Standard for antibodies (human) to *Salmonella enterica* Paratyphi A O2 polysaccharide**

As outlined above in section 8.2.6 enteric fever is endemic in South and South-East Asia, and several developing country vaccine manufacturers are producing O2 PS specific for paratyphoid to be combined with typhoid vaccines. At present, in-house or commercially available antisera and immunoassays are used to characterize the immunoreactivity of O2 PS. Given the potential importance of such vaccines in controlling enteric fever there is now a need for an international standard for human antibodies to *Salmonella enterica* serovar Paratyphi A O2 PS. It was therefore proposed that an international collaborative study be undertaken to establish a First WHO International Standard for antibodies (human) to *Salmonella enterica* Paratyphi A, O2 polysaccharide. Several vaccine manufacturers will be approached as potential sources of sera from volunteers taking part in Phase I clinical trials.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO International Standard for antibodies (human) to *Salmonella enterica* Paratyphi A O2 polysaccharide, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2018.

**8.2.8 Proposed First WHO Reference Panel for O-antigen polysaccharides for Shigella strains**

Shigella serotypes are a major global cause of morbidity and mortality. *Shigella flexneri* and *S. sonnei* are responsible for >125 million cases of moderate to severe diarrhoea cases per year, mostly in children <5 years of age. The majority
(90%) of infections caused by Shigella occur in sub-Saharan Africa and South Asia with travellers and deployed members of the military visiting endemic areas also at risk for shigellosis.

A number of organizations are now developing 4- to 6-valent vaccines to cover the immunodominant O-antigens of the most epidemiologically relevant serotypes (*S. sonnei* and *S. flexneri* 2a, 3a and 6). Monovalent vaccines for *S. sonnei* and *S. flexneri* 2a are also being developed with some already in early clinical trials. Given the common targets (the O-PS or repeating unit of LPS) of many of these vaccines, global standards are required for the quantification and testing of O-PS used in bioassays to measure the potency of these vaccines and to compare the immunogenicity of various vaccines. It was therefore proposed that an international collaborative study be undertaken to establish a First WHO Reference Panel for O-antigen polysaccharides for Shigella strains. Candidate materials would be of GMP standard and suitable for use in both immunoassays and physicochemical assays.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO Reference Panel for O-antigen polysaccharides for Shigella strains, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2019.

### 8.2.9 Proposed First WHO Reference Panel for anti-Shigella O-antigen polysaccharide serum (human)

As outlined above in section 8.2.8, Shigella serotypes are a major global cause of morbidity and mortality, and the 4- to 6-valent vaccines currently under development are intended to cover the immunodominant O-antigens of the most epidemiologically relevant serotypes (*S. sonnei*, *S. flexneri* 2a, 3a and 6). In the case of *S. sonnei* an O-antigen antibody level has been identified which is protective. At present, in-house or commercially available antisera and immunoassays are used to characterize vaccine immunoreactivity. Given the potential of a multivalent Shigella vaccine in controlling disease, there is now a need for international standards for human anti-O-antigen PS serum reflecting the most epidemiologically relevant serotypes. It was therefore proposed that an international collaborative study be undertaken to establish a First WHO Reference Panel for anti-Shigella O-antigen polysaccharide serum (human). Several vaccine manufacturers will be approached as potential sources of sera obtained from volunteers in Phase I clinical trials.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO Reference Panel for anti-Shigella O-antigen polysaccharide serum (human), and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2019.
8.2.10  Proposed First WHO International Standard for anti-\textit{Clostridium difficile} serum (human)

\textit{Clostridium difficile} infection (CDI) is caused by colonization of the human intestine by this Gram-positive bacterium, and can result in a high mortality rate. The risk of recurrence of CDI presents a burden to both patients and the health-care system with millions of new infections per year and thousands of deaths. CDI-associated disease constitutes a large majority of nosocomial diarrhoea cases in developed countries and is mediated by the effects of two secreted toxins – toxin A and toxin B. These toxins have key toxin-neutralizing epitopes that are the focus of current vaccine development studies.

Patients who develop strong anti-toxin antibody responses can clear \textit{C. difficile} infection and remain disease free, and several vaccines are currently in various stages of clinical trials. However, there are at present no international standards or reference reagents available to standardize the immune, functional or cytotoxic assays necessary for the evaluation of vaccine efficacy and quality.

As a human reference serum with an assigned unitage would be very useful in calibrating various immunoassays, it was proposed that an international collaborative study be undertaken to establish a First WHO International Standard for anti-\textit{Clostridium difficile} serum (human). Following testing of several batches of intravenous immunoglobulin, one manufacturer-donated batch was found to have high titres of antibodies to both toxin A and toxin B and would serve as a candidate material.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2015.2272) to develop a First WHO International Standard for anti-\textit{Clostridium difficile} serum (human), and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2017.
Annex 1

WHO Recommendations, Guidelines and other documents related to the manufacture and quality control of biological substances used in medicine

WHO Recommendations, Guidelines and other documents are intended to provide guidance to those responsible for the production of biological substances as well as to others who may have to decide upon appropriate methods of assay and control to ensure that products are safe, reliable and potent. WHO Recommendations (previously called Requirements) and Guidelines are scientific and advisory in nature but may be adopted by an NRA as national requirements or used as the basis of such requirements.

Recommendations concerned with biological substances used in medicine are formulated by international groups of experts and are published in the WHO Technical Report Series\(^1\) as listed below. A historical list of Requirements and other sets of Recommendations is available on request from the World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland.

Reports of the WHO Expert Committee on Biological Standardization published in the WHO Technical Report Series can be purchased from:

WHO Press
World Health Organization
20 avenue Appia
1211 Geneva 27
Switzerland
Telephone: + 41 22 791 3246
Fax: +41 22 791 4857
Email: bookorders@who.int
Website: www.who.int/bookorders

Individual Recommendations and Guidelines may be obtained free of charge as offprints by writing to:

Technologies Standards and Norms
Department of Essential Medicines and Health Products
World Health Organization
20 avenue Appia
1211 Geneva 27
Switzerland

\(^1\) Abbreviated in the following pages to “TRS”.

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<td>Recommendations, Guidelines and other documents</td>
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<td>Revised 1985, TRS 745 (1987)</td>
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<td>Adopted 2010, TRS 978 (2013)</td>
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<td>Adopted 2013, TRS 987 (2014)</td>
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<tr>
<td>Vaccines, prequalification procedure</td>
<td>Adopted 2010, TRS 978 (2013)</td>
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<td>Adopted 2006, TRS 962 (2011)</td>
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<td>Adopted 2015, TRS 999 (2016)</td>
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<td>Varicella vaccines (live)</td>
<td>Revised 1993, TRS 848 (1994)</td>
</tr>
<tr>
<td>Yellow fever vaccines</td>
<td>Revised 2010, TRS 978 (2013)</td>
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<tr>
<td>Yellow fever vaccines, laboratories approved by WHO for the production of</td>
<td>Revised 1995, TRS 872 (1998)</td>
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Annex 2

WHO good manufacturing practices for biological products
Replacement of Annex 1 of WHO Technical Report Series, No. 822

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Guidelines published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AEFI</td>
<td>adverse event following immunization</td>
</tr>
<tr>
<td>ATMP</td>
<td>advanced therapy medicinal product</td>
</tr>
<tr>
<td>BCG</td>
<td>bacille Calmette–Guérin</td>
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<tr>
<td>GMP</td>
<td>good manufacturing practice(s)</td>
</tr>
<tr>
<td>HEPA</td>
<td>high-efficiency particulate air</td>
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<tr>
<td>HVAC</td>
<td>heating, ventilation and air conditioning</td>
</tr>
<tr>
<td>IgE</td>
<td>immunoglobulin E</td>
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<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
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<tr>
<td>MCB</td>
<td>master cell bank</td>
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<tr>
<td>MSL</td>
<td>master seed lot</td>
</tr>
<tr>
<td>MVS</td>
<td>master virus seed</td>
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<tr>
<td>NRA</td>
<td>national regulatory authority</td>
</tr>
<tr>
<td>PDL</td>
<td>population doubling level</td>
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<tr>
<td>PQR</td>
<td>product quality review</td>
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<tr>
<td>PQS</td>
<td>pharmaceutical quality system</td>
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<tr>
<td>QRM</td>
<td>quality risk management</td>
</tr>
<tr>
<td>rDNA</td>
<td>recombinant DNA</td>
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<tr>
<td>SPF</td>
<td>specific pathogen free</td>
</tr>
<tr>
<td>TSE</td>
<td>transmissible spongiform encephalopathy</td>
</tr>
<tr>
<td>WCB</td>
<td>working cell bank</td>
</tr>
<tr>
<td>WSL</td>
<td>working seed lot</td>
</tr>
<tr>
<td>WVS</td>
<td>working virus seed</td>
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</tbody>
</table>
1. Introduction

Biological products can be defined according to their source material and method of manufacture. The source materials and methods employed in the manufacture of biological products for human use therefore represent critical factors in shaping their appropriate regulatory control. Biological products are derived from cells, tissues or microorganisms and reflect the inherent variability characteristic of living materials. The active substances in biological products are often too complex to be fully characterized by utilizing physicochemical testing methods alone and may show a marked heterogeneity from one preparation and/or batch to the next. Consequently, special considerations are needed when manufacturing biological products in order to maintain consistency in product quality.

Good manufacturing practices (GMP) for biological products were first published by WHO in 1992 (1). This current revision reflects subsequent developments that have taken place in science and technology, and in the application of risk-based approaches to GMP (2–14). The content of this document should be considered complementary to the general recommendations set out in the current WHO good manufacturing practices for pharmaceutical products: main principles (2) and in other WHO documents related specifically to the production and control of biological products.

This document is intended to serve as a basis for establishing national guidelines for GMP for biological products. If a national regulatory authority (NRA) so desires, the guidance provided may be adopted as definitive national requirements, or modifications may be justified and made by the NRA in light of the risk–benefit balance and legal considerations in each authority. In such cases, it is recommended that any modification to the principles and technical specifications set out below should be made only on the condition that the modifications ensure product quality, safety and efficacy that are at least equivalent to that recommended in this document.

2. Scope

The guidance provided in this document applies to the manufacture, control and testing of biological products for human use – from starting materials and preparations (including seed lots, cell banks and intermediates) to the finished product.

Manufacturing procedures within the scope of this document include:

- growth of strains of microorganisms and eukaryotic cells;
- extraction of substances from biological tissues, including human, animal and plant tissues, and fungi;
- recombinant DNA (rDNA) techniques;
- hybridoma techniques;
- propagation of microorganisms in embryos or animals.

Medicinal products of biological origin manufactured by these procedures include allergens, antigens, vaccines, certain hormones, cytokines, monoclonal antibodies (mAbs), enzymes, animal immune sera, products of fermentation (including products derived from rDNA), biological diagnostic reagents for in vivo use and advanced therapy medicinal products (ATMPs) used for example in gene therapy and cell therapy.

For human whole blood, blood components and plasma-derived products for therapeutic use separate comprehensive WHO guidance is available and should be followed (12, 15).

In some countries certain small-molecule medicinal products (for example, antibiotics) are not defined as biological products. Nevertheless, where the manufacturing procedures described in this document are used then the guidance provided may be followed.

The preparation of investigational medicinal products for use in clinical trials should follow the basic principles of GMP set out in these and other WHO GMP guidelines (2, 16) as appropriate. However, certain other requirements (such as process and analytical method validations) could be completed before marketing authorization (17–19).

The current document does not provide detailed recommendations for specific classes of biological products (for example, vaccines). Attention is therefore directed to other relevant WHO documents, and in particular to WHO recommendations to assure the quality, safety and efficacy of specific products.¹

Table 1 illustrates the typical risk-based application of the current document (4, 7). It should be noted that this table is illustrative only and is not intended to describe the precise scope.

Table 1
Scope of the current document (illustrative)

<table>
<thead>
<tr>
<th>Type and source of material</th>
<th>Example products</th>
<th>Application of this document to steps in manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Animal or plant sources: non-transgenic</td>
<td>Heparins, insulin, enzymes, proteins, allergen extract, ATMPs, animal immune sera</td>
<td>Collection of plant, organ, tissue or fluid</td>
</tr>
<tr>
<td>2. Virus or bacteria/fermentation/cell culture</td>
<td>Viral or bacterial vaccines, enzymes, proteins</td>
<td>Establishment and maintenance of MCB, WCB, MSL/WVS</td>
</tr>
<tr>
<td>3. Biotechnology fermentation/cell culture</td>
<td>Recombinant products, mAbs, allergens, vaccines, gene therapy (viral and non-viral vectors, plasmids)</td>
<td>Establishment and maintenance of MCB, WCB, MSL, WSL</td>
</tr>
<tr>
<td>4. Animal sources: transgenic</td>
<td>Recombinant proteins, ATMPs</td>
<td>Master and working transgenic bank</td>
</tr>
<tr>
<td>5. Plant sources: transgenic</td>
<td>Recombinant proteins, vaccines, allergens</td>
<td>Master and working transgenic bank</td>
</tr>
<tr>
<td>Type and source of material</td>
<td>Example products</td>
<td>Application of this document to steps in manufacture</td>
</tr>
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<td>-----------------------------</td>
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<tr>
<td>6. Human sources</td>
<td>Urine-derived enzymes, hormones</td>
<td>Collection of fluid</td>
</tr>
<tr>
<td>7. Human and/or animal sources</td>
<td>Gene therapy: genetically modified cells</td>
<td>Donation, procurement and testing of starting tissue/cells&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Somatic cell therapy</td>
<td>Donation, procurement and testing of starting tissue/cells&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Tissue-engineered products</td>
<td>Donation, procurement and testing of starting tissue/cells&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> GMP guidelines, as described in this document, are not applied to this step. Other national regulations, requirements, recommendations and/or guidelines may apply as deemed necessary by the NRA.

MCB = master cell bank; MSL = master seed lot; MVS = master virus seed; WCB = working cell bank; WSL = working seed lot; WVS = working virus seed.
3. Terminology

In addition to the terms defined in WHO good manufacturing practices for pharmaceutical products: main principles (2) and WHO good manufacturing practices for sterile pharmaceutical products (3), the definitions given below apply to the terms as used in the current document. These terms may have different meanings in other contexts.

**Active substance:** a defined process intermediate containing the active ingredient, which is subsequently formulated with excipients to produce the drug product. This may also be referred to as “drug substance” or “active ingredient” in other documents.

**Adventitious agents:** contaminating microorganisms of the cell culture or source materials, including bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents and viruses that have been unintentionally introduced into the manufacturing process of a biological product. The source of these contaminants may be the legacy of the cell line, or the raw materials used in the culture medium to propagate the cells (in banking, in production or in their legacy), the environment, personnel, equipment or elsewhere.

**Allergen:** a molecule capable of inducing an immunoglobulin E (IgE) response and/or a Type I allergic reaction.

**Antibodies:** proteins produced naturally by the B-lymphocytes that bind to specific antigens. Using rDNA technology antibodies are also produced in other (continuous) cell lines. Antibodies may be divided into two main types – monoclonal and polyclonal antibodies – based on key differences in their methods of manufacture. Also called immunoglobulins.

**Antigens:** substances (for example, toxins, foreign proteins, bacteria, tissue cells and venoms) capable of inducing specific immune responses.

**Axenic:** a single organism in culture which is not contaminated with any other organism.

**Bioburden:** the level and type (objectionable or not) of microorganisms present in raw materials, media, biological substances, intermediates or finished products. Regarded as contamination when the level and/or type exceed specifications.

**Biohazard:** any biological material considered to be hazardous to people and/or the environment.

**Biological starting materials:** starting materials derived from a biological source that mark the beginning of the manufacturing process of a drug, as described in a marketing authorization or licence application, and from which the active ingredient is derived either directly (for example, plasma derivatives, ascitic fluid and bovine lung) or indirectly (for example, cell substrates, host/vector production cells, eggs and viral strains).
**Biosafety risk group:** denotes the containment conditions required for safe handling of organisms associated with different hazards, ranging from Risk Group 1 (lowest risk, no or low individual and community risk, and unlikely to cause disease) to Risk Group 4 (highest risk, high individual and community risk, usually causes severe disease, and which is likely to spread with no prophylaxis or treatment available) (20).

**Campaign manufacture:** the manufacture of an uninterrupted sequence of batches of the same product or intermediate in a given time period, followed by strict adherence to accepted control measures before switching to another product or different serotype. The different products are not run at the same time but may be run on the same equipment.

**Cell bank:** a collection of appropriate containers whose contents are of uniform composition and stored under defined conditions. Each container represents an aliquot of a single pool of cells.

**Cell culture:** the process by which cells that are no longer organized into tissues are grown in vitro under defined and controlled conditions. Cell cultures are operated and processed under axenic conditions to ensure a pure culture absent of microbial contamination.

**Cell stock:** primary cells expanded to a given number of cells to be aliquoted and used as starting material for production of a limited number of lots of a cell-based medicinal product.

**Containment:** the concept of using a process, equipment, personnel, utilities, system and/or facility to contain product, dust or contaminants in one zone, preventing them from entering into another zone and/or escaping.

**Continuous culture:** a process by which the growth of cells is maintained by periodically replacing a portion of the cells and the medium so that there is no lag or saturation phase.

**Control strategy:** a planned set of controls derived from current product and process understanding that assures process performance and product quality. The controls can include parameters and attributes related to active substance and finished product materials and components; facility and equipment operating conditions; in-process controls; finished product specifications; and the associated methods and frequency of monitoring and control.

**Cross-contamination:** contamination of a starting material, intermediate product or finished product with another starting material or product during production. In multi-product facilities, cross-contamination can occur throughout the manufacturing process, from generation of the master cell bank (MCB) and working cell bank (WCB) to finished product.

**Dedicated:** facility, personnel, equipment or piece of equipment used only in the manufacture of a particular product or group of specified products of similar risk.
Dedicated area: an area that may be in the same building as another area but which is separated by a physical barrier and which has, for example, separate entrances, staff facilities and air-handling systems. Also referred to as “self-contained facility” in other GMP documents.

Feeder cells: cells used in co-culture to maintain pluripotent stem cells. For human embryonic stem cell culture, typical feeder layers include mouse embryonic fibroblasts or human embryonic fibroblasts that have been treated to prevent them from dividing.

Finished product: a finished dosage form that has undergone all stages of manufacture, including packaging in its final container and labelling. Also referred to as “finished dosage form”, “drug product” or “final product” in other documents.

Fermentation: maintenance or propagation of microbial cells in vitro (fermenter). Fermentation is operated and progressed under axenic conditions to ensure a pure culture absent of contaminating microorganisms.

Harvesting: the procedure by which the cells, inclusion bodies or crude supernatants containing the unpurified active ingredient are recovered.

Hybridoma: an immortalized cell line that secretes desired (monoclonal) antibodies and which is typically derived by fusing B-lymphocytes with tumour cells.

Inactivation: removal or reduction to an acceptable limit of infectivity of microorganisms or detoxification of toxins by chemical or physical modification.

Master cell bank (MCB): a quantity of well-characterized cells of animal or other origin, derived from a cell seed at a specific population doubling level (PDL) or passage level, dispensed into multiple containers and stored under defined conditions. The MCB is prepared from a single homogeneously mixed pool of cells. In some cases, such as genetically engineered cells, the MCB may be prepared from a selected cell clone established under defined conditions. However, the MCB may not be clonal. The MCB is used to derive a working cell bank (WCB).

Monoclonal antibodies (mAbs): homogenous antibody population obtained from a single clone of lymphocytes or by recombinant technology and which bind to a single epitope.

Pharmaceutical quality system (PQS): management system used by a pharmaceutical company to direct and control its activities with regard to quality.

Polyclonal antibodies: antibodies derived from a range of lymphocyte clones and produced in humans and animals in response to the epitopes on most “non-self” molecules.

Primary containment: a system of containment that prevents the escape of a biological agent into the immediate working environment. It involves the use of closed containers or biological safety cabinets along with secure operating procedures.
Quality risk management (QRM): a systematic process for the assessment, control, communication and review of risks to the quality of pharmaceutical products across the product life-cycle.

Reference sample: a sample of a batch of starting material, packaging material, intermediate or finished product which is stored for the purpose of being analysed should the need arise during the shelf-life of the batch concerned.

Retention sample: a sample of a fully packaged unit from a batch of finished product. It is stored for identification purposes (for example, of presentation, packaging, labelling, patient information leaflet, batch number and expiry date) should the need arise during the shelf-life of the batch concerned.

Seed lot: a quantity of live cells or viruses which has been derived from a single culture (though not necessarily clonal), has a uniform composition and is aliquoted into appropriate storage containers from which all future products will be derived, either directly or via a seed lot system. The following derived terms are used in this document – master seed lot (MSL): a lot or bank of cells or viruses from which all future vaccine production will be derived. The MSL represents a well-characterized collection of cells or viruses or bacteria of uniform composition. Also referred to as “master virus seed” (MVS) for virus seeds, “master seed bank”, “master seed antigen” or “master transgenic bank” in other documents; and working seed lot (WSL): a cell or viral or bacterial seed lot derived by propagation from the MSL under defined conditions and used to initiate production of vaccines on a lot-by-lot basis. Also referred to as “working virus seed” (WVS) for virus seeds, “working seed bank”, “working seed antigen” or “working transgenic bank” in other documents.

Specific pathogen free (SPF): denoting animals or animal materials (such as chickens, embryos, eggs or cell cultures) derived from groups of animals (for example, flocks or herds) free from specified pathogens, and used for the production or quality control of biological products. Such flocks or herds are defined as animals sharing a common environment and having their own caretakers who have no contact with non-SPF groups.

Starting materials: any substances of a defined quality used in the production of a pharmaceutical product, but excluding packaging materials. In the context of biological products manufacturing, examples of starting materials may include cryo-protectants, feeder cells, reagents, growth media, buffers, serum, enzymes, cytokines, growth factors and amino acids.

Transgenic: denoting an organism that contains a foreign gene in its normal genetic component for the expression of biological pharmaceutical materials.

Vaccine: a preparation containing antigens capable of inducing an active immune response for the prevention, amelioration or treatment of infectious diseases.
Working cell bank (WCB): a quantity of well-characterized cells of animal or other origin, derived from an MCB at a specific PDL or passage level, dispensed into multiple containers and stored under defined conditions. The WCB is prepared from a single homogeneously mixed pool of cells (often, this is the MCB). One or more of the WCB containers is used for each production culture.

4. Principles and general considerations

The manufacture of biological products should be undertaken in accordance with the basic principles of GMP. The points covered by the current document should, therefore, be considered as complementary to the general recommendations set out in the current WHO good manufacturing practices for pharmaceutical products: main principles (2) and associated specialized guidelines and recommendations (3, 4, 10, 13, 14) as well as other WHO documents related specifically to the production and control of biological products established by the WHO Expert Committee on Biological Standardization.²

The manufacture, control and administration of biological active substances and finished products require certain specific considerations and precautions arising from the nature of these products and their processes. Unlike conventional pharmaceutical products which are manufactured using chemical and physical techniques capable of a high degree of consistency, the manufacture of biological active substances and finished products involves biological processes and materials, such as cultivation of cells or extraction from living organisms. As these biological processes may display inherent variability, the range and nature of by-products may also be variable. As a result, quality risk management (QRM) principles are particularly important for this class of materials and should be used to develop the control strategy across all stages of manufacture so as to minimize variability and reduce the opportunity for contamination and cross-contamination.

Materials and processing conditions used in cultivation processes are designed to provide conditions for the growth of target cells and microorganisms – therefore, extraneous microbial contaminants have the opportunity to grow. Furthermore, many biological products have limited ability to withstand certain purification techniques, particularly those designed to inactivate or remove adventitious viral contaminants. The design of the processes, equipment, facilities, utilities, the conditions of preparation and addition of buffers and reagents, sampling, and training of the operators are key considerations in minimizing

such contamination events. Specifications outlined in WHO guidelines and recommendations will determine whether and to what stage of production substances and materials can have a defined level of bioburden or need to be sterile. Similarly, manufacturing should be consistent with other specifications set out in the product summary files, marketing authorization or clinical trial approvals (for example, number of generations (expressed as doublings or passages) between the seed lot or cell bank and the finished product).

Many biological materials (such as live-attenuated bacteria and viruses) cannot be terminally sterilized by heat, gas or radiation. In addition, some products, such as certain live and adjuvanted vaccines (for example, bacille Calmette–Guérin (BCG) or cholera), may not be sterilized by filtration processes. For these axenic products, processing should be conducted aseptically to minimize the introduction of contaminants from the point where a potential contamination cannot be removed from the manufacturing process. Relevant WHO documents should be consulted on the validation of specific manufacturing steps such as virus removal or inactivation (21). Robust environmental controls and monitoring and, wherever feasible, in situ cleaning and sterilization systems, together with the use of closed systems can significantly reduce the risk of accidental contamination and cross-contamination.

Control usually involves biological analytical techniques, which typically have a greater variability than physicochemical determinations. The combination of variability in starting materials and the potential for subtle changes during the manufacturing process of biological products also requires an emphasis on production consistency. This is of particular concern because of the need to link consistency to original clinical trials documenting the product’s safety and efficacy. A robust manufacturing process is therefore crucial and in-process controls take on a particular importance in the manufacture of biological active substances and medicinal products.

Because of the risks inherent in producing and manipulating pathogenic and transmissible microorganisms during the production and testing of biological materials, GMP should prioritize the safety of the recipient to whom the biological product is administered, the safety of personnel during operation and the protection of the environment.

Biosafety considerations should follow national guidelines and (if applicable and available) international guidelines. In most countries, the regulation of GMP and biosafety are governed by different institutions. In the context of manufacturing pathogenic biological products of Biosafety Risk Group 3 and 4, close collaboration between such institutions is especially required to assure that both product contamination and environmental contamination levels are controlled within acceptable limits. Specific recommendations regarding containment are outlined below in section 10.
5. Pharmaceutical quality system and quality risk management

Biological products, like any pharmaceutical product, should be manufactured in accordance with the requirements of a pharmaceutical quality system (PQS) based on a life-cycle approach as defined in WHO good manufacturing practices for pharmaceutical products: main principles (2). This approach facilitates innovation and continual improvement, and also strengthens the link between pharmaceutical development and manufacturing activities.

QRM principles should be used to develop the control strategy across all manufacturing and control stages – including materials sourcing and storage, personnel and materials flow, manufacture and packaging, quality control, quality assurance, storage and distribution activities, as described in relevant WHO guidelines (14) and other documents (22). Due to the inherent variability of biological processes and starting materials, ongoing trend analysis and periodic review are particularly important elements of PQS. Thus, special attention should be paid to starting material controls, change control, trend analysis and deviation management in order to ensure production consistency. Monitoring systems should be designed so as to provide early detection of any unwanted or unanticipated factors that may affect the quality, safety and efficacy of the product. The effectiveness of the control strategy in monitoring, reducing and managing such risks should be regularly reviewed and the systems updated as required taking into account scientific and technical progress.

6. Personnel

6.1 Personnel responsible for production and control should have an adequate background in relevant scientific disciplines such as microbiology, biology, biometry, chemistry, medicine, pharmacy, pharmacology, virology, immunology, biotechnology and veterinary medicine, together with sufficient practical experience to enable them to perform their duties.

6.2 The health status of personnel should be taken into consideration as part of ensuring product safety. Where necessary, personnel engaged in production, maintenance, testing and animal care (and inspections) should be vaccinated with appropriate specific vaccines and have regular health checks. Any changes in the health status of personnel which could adversely affect the quality of the product should preclude their working in the production area, and appropriate records kept. The scope and frequency of health monitoring should be commensurate with the risk to the product and personnel.
6.3 Training in cleaning and disinfection procedures, hygiene and microbiology should emphasize the risk of microbial and adventitious contamination and the nature of the target microorganisms and growth media routinely used.

6.4 Where required to minimize the opportunity for cross-contamination, restrictions on the movement of all personnel (including quality control, maintenance and cleaning staff) should be defined on the basis of QRM principles. In general, all personnel including those not routinely involved in the production operation (such as management, engineering staff and validation staff or auditors) should not pass from areas with exposure to live microorganisms, genetically modified microorganisms, animal tissue, toxins, venoms or animals to areas where other products (inactivated or sterile) or different organisms are handled. If such passage is unavoidable during a working day, then contamination control measures (for example, clearly defined decontamination measures such as a complete change of appropriate clothing and shoes, and showering if applicable) should be followed by all personnel visiting any such production area unless otherwise justified on the basis of QRM.

6.5 Because the risks are difficult to manage, personnel working in an animal facility should be restricted from entering production areas where potential risks of cross-contamination exist.

6.6 Staff assigned to the production of BCG products should not work with other infectious agents. In particular, they should not work with virulent strains of *Mycobacterium tuberculosis*, nor should they be exposed to a known risk of tuberculosis infection (23). Additionally, they should be carefully monitored, with regular health checks that screen for tuberculosis infection.

6.7 If personnel working in BCG manufacturing and in animal quarters need to be reassigned to other manufacturing units they should not be allowed into such units until they pass their health check.

7. Starting materials

7.1 The source, origin and suitability of active substances, starting materials (for example, cryo-protectants and feeder cells), buffers and media (for example, reagents, growth media, serum, enzymes, cytokines, growth factors and amino acids) and other components of the finished product should be clearly defined and controlled according to the principles set out in WHO guidance on GMP for pharmaceutical products (2).
7.2 Manufacturers should retain information describing the source and quality of the biological materials used for at least 1 year after the expiry date of the finished products and according to local regulations concerning biological products. It has been found that documents retained for longer periods may provide useful information related to adverse events following immunization (AEFIs) and other investigations.

7.3 All starting material suppliers (that is, manufacturers) should be initially qualified on the basis of documented criteria and a risk-based approach. Regular assessments of their status should also be carried out. Particular attention should be given to the identification and monitoring of any variability that may affect biological processes. When starting materials are sourced from brokers who could increase the risk of contamination by performing repackaging operations under GMP (2, 4) they should be carefully qualified; an audit may form part of such qualification, as needed.

7.4 An identity test, or equivalent, should be performed on each batch of received starting materials prior to release. The number of containers sampled should be justified on the basis of QRM principles and in agreement with all applicable guidelines (2). The identification of all starting materials should be in compliance with the requirements appropriate to the stage of manufacture. The level of testing should be commensurate with the qualification level of the supplier and the nature of the materials used. In the case of starting material used to manufacture active substances the number of samples taken should be based on statistically recognized criteria and QRM principles (2). However, for starting materials and intermediates used in the formulation of finished product each container should be sampled for identity testing in accordance with the main principles of GMP for pharmaceutical products unless reduced testing has been validated.

7.5 The sampling process should not adversely affect the quality of the product. Incoming starting materials should be sampled under appropriate conditions in order to prevent contamination and cross-contamination.

7.6 Where justified (such as the special case of sterile starting materials) it may be acceptable to reduce the risk of contamination by not performing sampling at the time of receipt but to perform the testing later on samples taken at the time of use. In such cases, release of the finished product is conditional upon satisfactory results of these tests.

7.7 Where the necessary tests for approving starting materials take a significantly long time, it may be permissible by exception to process starting materials before the test results are available. The use of these materials should be clearly justified in a documented manner, and the risks
should be understood and assessed under the principles of QRM. In such cases, release of the finished product is conditional upon satisfactory results from the tests. It must be ensured that this is not standard practice and occurs only with justification of the risk taken.

7.8 The risk of contamination of starting materials during their passage along the supply chain should be assessed, with particular emphasis on adventitious agents such as those causing TSEs (24). Other materials that come into direct contact with manufacturing equipment and/or with potential product contact surfaces (such as filter media, growth media during aseptic process simulations and lubricants) should also be controlled. A quality risk assessment should be performed to evaluate the potential for adventitious agents in biological starting materials.

7.9 Where required, the sterilization of starting materials should be carried out by heat whenever possible. Where necessary, other appropriate validated methods may also be used for this purpose (such as irradiation and filtration).

7.10 The controls required for ensuring the quality of sterile starting materials and of the aseptic manufacturing process should be based on the principles and guidance contained in the current WHO good manufacturing practices for sterile pharmaceutical products (3).

7.11 The transport of critical materials, reference materials, active substances, human tissues and cells to the manufacturing site should be controlled as part of a written quality agreement between the responsible parties if they are different commercial entities. Manufacturing sites should have documentary evidence of adherence to the specified storage and transport conditions, including cold chain requirements, if required. The required traceability – starting at tissue establishments through to the recipient(s), and including the traceability of materials in contact with the cells or tissues – should be ensured, maintained and documented.

8. Seed lots and cell banks

8.1 The recommendations set out in WHO good manufacturing practices for active pharmaceutical ingredients (4) should be followed – specifically section 18 on specific guidance for active pharmaceutical ingredients manufactured by cell culture/fermentation.

8.2 Where human or animal cells are used as feeder cells in the manufacturing process, appropriate controls over their sourcing, testing, transport and storage should be in place.
8.3 In order to prevent the unwanted drift of genetic properties which might result from repeated subcultures or multiple generations, the production of biological products obtained by microbial culture, cell culture or propagation in embryos and animals should be based on a system of master and working seed lots and/or cell banks; which is the beginning of the manufacturing process of certain biological products (for example, vaccines).

8.4 The number of generations (expressed as passages or doublings) between the seed lot or cell bank and the finished product, defined as maximum, should be consistent with the marketing authorization dossier and should not be exceeded.

8.5 Cell-based medicinal products are often generated from a cell stock obtained from a limited number of passages. In contrast with the two-tier system of MCBs and WCBs, the number of production runs from a cell stock is limited by the number of aliquots obtained after expansion and does not cover the entire life-cycle of the product. Cell stock changes should be covered by a validation protocol and communicated to the NRA, as applicable.

8.6 Establishment and handling of the MCBs and WCBs should be performed under conditions which are demonstrably appropriate. These should include an appropriately controlled environment to protect the seed lot and the cell bank, and the personnel handling them. To establish the minimum requirements for clean room grade and environmental monitoring in the case of vaccines see the WHO Environmental monitoring of clean rooms in vaccine manufacturing facilities: points to consider for manufacturers of human vaccines (25). During the establishment of the seed lot and cell bank, no other living or infectious material (such as viruses, cell lines or microbial strains) should be handled simultaneously in the same area or by the same persons, as set out in current WHO Recommendations (26).

8.7 Quarantine and release procedures for master and working cell banks/seed lots should be followed, including adequate characterization and testing for contaminants. Initially, full characterization testing of the MCB should be done, including genetic identification. A new MCB (from a previous initial clone, MCB or WCB) should be subjected to the same established testing as the original MCB, unless otherwise justified. Thereafter, the viability, purity and other stability-indicating attributes of seed lots and cell banks should be checked regularly according to justified criteria. Evidence of the stability and recovery of the seed lots and banks should be documented and records should be kept in a manner that permits trend evaluation.
8.8 Each storage container should be adequately sealed, clearly labelled and kept at an appropriate temperature. A stock inventory should be kept. The storage temperature should be recorded continuously and, where applicable, the liquid nitrogen level should be monitored. Any deviation from the set limits, and any corrective and preventive action taken, should be recorded. Temperature deviations should be detected as early as possible (for example, through the use of an alarm system for temperature and nitrogen levels).

8.9 Seed lots and cell banks should be stored and used in such a way as to minimize the risks of contamination or alteration (for example, stored in qualified ultra-low temperature freezers or liquid nitrogen storage containers). Control measures for the storage of different seeds and/or cells in the same area or equipment should prevent mix-up and should take into account the infectious nature of the materials in order to prevent cross-contamination.

8.10 MSLs, MCBs, and preferably also WSLs and WCBs, should be stored in two or more controlled separate sites in order to minimize the risk of total loss due to natural disaster, equipment malfunction or human error. A contingency plan should be in place.

8.11 The storage and handling conditions for the cell or seed banks should be defined. Access should be controlled and restricted to authorized personnel, and appropriate access records maintained. Records of location, identity and inventory of individual containers should also be kept. Once containers are removed from the seed lot/cell bank management system they should not be returned to stock.

9. Premises and equipment

9.1 In general, preparations containing live microorganisms or live viruses should not be manufactured and containers should not be filled in areas used for the processing of other pharmaceutical products. However, if the manufacturer can demonstrate and validate effective containment and decontamination of the live microorganisms and viruses then the use of multi-product facilities may be justifiable. In such cases, measures such as campaign production, closed systems and/or disposable systems should be considered and should be based on QRM principles (see sections 10 and 13 below on containment and campaign production respectively).

9.2 Documented QRM should be carried out for every additional product in a biological manufacturing multi-product facility, which may include a potency and toxicological evaluation based on cross-contamination
risks. Other factors to be taken into account include facility/equipment design and use, personnel and material flows, microbiological controls, physicochemical characteristics of the active substance, process characteristics, cleaning processes and analytical capabilities relative to the relevant limits established from product evaluation. The outcome of the QRM process should be the basis for determining the necessity for premises and equipment to be dedicated to a particular product or product family, and the extent to which this should be the case. This may include dedicating specific product-contact parts. The NRA should approve the use of a manufacturing facility for the production of multiple products on case-to-case basis.

9.3 Killed vaccines, antisera and other biological products – including those made by rDNA techniques, toxoids and bacterial extracts – may, following inactivation, be manufactured on the same premises provided that adequate decontamination and cleaning measures are implemented on the basis of QRM.

9.4 Cleaning and sanitization should take into account the fact that processes often include the handling of growth media and other growth-promoting agents. Validation studies should be carried out to ensure the effectiveness of cleaning, sanitization and disinfection, including elimination of residues of used agents. Environmental and personnel safety precautions should be taken during the cleaning and sanitization processes. The use of cleaning and sanitizing agents should not pose any major risk to the performance of equipment.

The use of closed systems to improve asepsis and containment should be considered where practicable. Where open systems are utilized during processing (for example, during addition of growth supplements, media, buffers and gases, and during sampling and aseptic manipulations during the handling of live cells such as in cell-therapy products) control measures should be put in place to prevent contamination, mix-up and cross-contamination. Logical and unidirectional flows of personnel, materials and processes, and the use of clean-in-place and sterilize-in-place systems, should be considered wherever possible. Where sterile single-use systems such as bags and connectors are utilized, they should be qualified with respect to suitability, extractables, leachables and integrity.

9.5 Because of the variability of biological products, and of the corresponding manufacturing processes, approved starting materials that have to be measured or weighed for the production process (such as growth media, solutions and buffers) may be kept in small stocks in the production area for a specified period of time according to defined criteria – such as for
the duration of manufacture of the batch or of the campaign. Appropriate storage conditions and controls should be maintained during such temporary storage. These materials should not be returned to the general stock. Materials used to formulate buffers, growth media and so on should be weighed and made into a solution in a contained area using local protection (such as a classified weighing booth) and outside the aseptic processing areas in order to minimize particulate contamination of the latter.

9.6 In manufacturing facilities, the mix-up of entry and exit of personnel should be avoided through the use of separate changing rooms or through procedural controls where Biosafety Risk Group 3 or 4 organisms are handled (20).

10. **Containment**

10.1 Airborne dissemination of live microorganisms and viruses used for the production process, including those from personnel, should be avoided.

10.2 Adequate precautions should be taken to avoid contamination of the drainage system with dangerous effluents. Drainage systems should be designed in such a way that effluents can be effectively neutralized or decontaminated to minimize the risk of cross-contamination. Specific and validated decontamination systems should be considered for effluents when infectious and/or potentially infectious materials are used for production. Local regulations should be complied with in order to minimize the risk of contamination of the external environment according to the risk associated with the biohazardous nature of waste materials.

10.3 Dedicated production areas should be used for the handling of live cells capable of persistence in the manufacturing environment, for pathogenic organisms of Biosafety Risk Group 3 or 4 and/or for spore-forming organisms until the inactivation process is accomplished and verified. For *Bacillus anthracis*, *Clostridium tetani* and *Clostridium botulinum* strictly dedicated facilities should be utilized for each individual product. Up-to-date information on these and other high-risk or “special” agents should be sought from major information resources (27). Where campaign manufacture of spore-forming organisms occurs in a facility or suite of facilities only one product should be processed at any one time.

Use of any pathogenic organism above Biosafety Risk Group 3 may be permitted by the NRA according to the biohazard classification of the organism, the risk assessment of the biological product and its emergency demand.
10.4 Production of BCG-related product should take place in a dedicated area and by means of dedicated equipment and utilities (such as heating, ventilation and air conditioning (HVAC) systems) in order to minimize the hazard of cross-contamination.

10.5 Specific containment requirements apply to poliomyelitis vaccine in accordance with the WHO global action plan to minimize poliovirus facility-associated risk (28) and with WHO Guidelines for the safe production and quality control of inactivated poliomyelitis vaccine manufactured from wild polioviruses (29). The measures and procedures necessary for containment (that is, for protecting the environment and ensuring the safety of the operator) should not conflict with those for ensuring product quality.

10.6 Air-handling systems should be designed, constructed and maintained to minimize the risk of cross-contamination between different manufacturing areas as required. The need for dedicated air-handling units or single-pass systems should be based on QRM principles, taking into account the biohazard classification and containment requirements of the relevant organism, and process and equipment risks. In the case of Biosafety Risk Group 3 organisms, air should not be recirculated to any other area in the facility and should be exhausted through high-efficiency particulate air (HEPA) filters that are regularly checked for performance. A dedicated non-recirculating ventilation system and HEPA-filtering of exhaust air are required when handling Biosafety Risk Group 4 organisms (27).

10.7 Primary containment equipment should be designed and initially qualified for integrity in order to ensure that the escape of biological agents and/or material into the immediate working area and outside environment is prevented. Thereafter, in line with relevant guidelines and QRM principles, periodical tests should be performed to ensure that the equipment is in proper working condition.

10.8 Activities associated with the handling of live biological agents (such as centrifugation and blending of products which can lead to aerosol formation) should be contained in such a way as to prevent contamination of other products or the egress of live agents into the working and/or outside environment. The viability of such organisms and their biohazard classification should be taken into consideration as part of the management of such risks.

Accidental spillages, especially of live organisms, must be dealt with quickly and safely. Validated decontamination measures should be
available for each organism or groups of related organisms. Where different strains of a single bacteria species or very similar viruses are involved, the decontamination process may be validated with one representative strain, unless the strains vary significantly in their resistance to the decontaminating agent(s) used.

10.9 Areas where Biosafety Risk Group 3 or 4 organisms are handled should always have a negative air pressure relative to the environment. This will ensure the containment of the organism in unlikely events such as failure of the door interlock. Air-lock doors should be interlocked to prevent them being opened simultaneously. Differential pressure alarms should be present wherever required, and should be validated and monitored.

10.10 Air-vent filters should be hydrophobic and subject to integrity testing at intervals determined by a QRM approach.

10.11 Where the filtration of exhaust air is necessary, the safe changing of filters should be ensured or bag-in-bag-out housings should be employed. Once removed, filters should be decontaminated and properly destroyed. In addition to HEPA filtration other inactivation technologies such as heat inactivation and steam scavenging may be considered for exhaust air to ensure effective inactivation of pathogenic organisms of Biosafety Risk Group 3 and/or 4.

11. Clean rooms

11.1 The WHO good manufacturing practices for sterile pharmaceutical products (3) defines and establishes the required class/grade of clean areas for the manufacture of sterile products according to the operations performed, including final aseptic fill. Additionally, in order to address the specific manufacturing processes involved in the production of biological products, and particularly vaccines, the WHO Environmental monitoring of clean rooms in vaccine manufacturing facilities: points to consider for manufacturers of human vaccines (25) guidance document may be used to develop the environmental classification requirements for biological manufacturing processes.

As part of the control strategy, the degree of environmental control of particulate and microbial contamination of the production premises should be adapted to the intermediate or finished product, and also to the production step, taking into account the potential level of contamination of the starting materials and the risks to the finished product.
11.2 The environmental monitoring programme should be supplemented with methods to detect the presence of the specific microorganisms used for production (for example, recombinant yeast and toxin- or polysaccharide-producing bacteria). The environmental monitoring programme may also include detection of the produced organisms and adventitious agents of production organisms, especially when campaign manufacture is applied on the basis of QRM principles.

12. Production

12.1 Since cultivation conditions, media and reagents are designed to promote the growth of cells or microbial organisms, typically in an axenic state, particular attention should be paid to the control strategy for ensuring that effective steps are in place for preventing or minimizing the occurrence of unwanted bioburden, endotoxins, viruses of animal and human origin, and associated metabolites.

12.2 The QRM process should be the basis for implementing the technical and organizational measures required to control the risks of contamination and cross-contamination. These could include, though are not limited to:

- carrying out processing and filling in segregated areas;
- containing material transfer by means of an airlock and appropriate type of pass box with validated transfer procedures, clothing change and effective washing and decontamination of equipment;
- recirculation of only treated (HEPA-filtered) air;
- acquiring knowledge of the key characteristics (for example, pathogenicity, detectability, persistence and susceptibility to inactivation) of all cells, organisms and any adventitious agents within the same facility;
- when considering the acceptability of concurrent work in cases where production is characterized by multiple small batches from different starting materials (for example, cell-based products) taking into account factors such as the health status of donors and the risk of total loss of a product from or for specific patients during development of the cross-contamination control strategy;
- preventing the risk of live organisms and spores entering non-related areas or equipment by addressing all potential routes of cross-contamination (for example, through the HVAC system) through the use of single-use components and closed systems;
- conducting environmental monitoring specific to the microorganism being manufactured in adjacent areas while paying attention to cross-contamination risks arising from the use of certain monitoring equipment (such as that used for airborne particle monitoring) in areas handling live and/or spore-forming organisms;
- using campaign-based production (see section 13 below).

12.3 When applicable, the inoculum preparation area should be designed so as to effectively control the risk of contamination, and should be equipped with a biosafety hood for primary containment.

12.4 If possible, growth media should be sterilized in situ by heat or in-line microbial-retentive filters. Additionally, in-line microbial-retentive filters should be used for the routine addition of gases, media, acids, alkalis and so on to fermenters or bioreactors.

12.5 Data from continuous monitoring of certain production processes (such as fermentation) should form part of the batch record. Where continuous culture is used, special consideration should be given to parameters such as temperature, pH, pO2, CO2 and the rate of feed or carbon source with respect to growth of cells.

12.6 In cases where a viral inactivation or removal process is performed, measures should be taken (for example, in relation to facility layout, unidirectional flow and equipment) to avoid the risk of recontamination of treated products by non-treated products.

12.7 A wide variety of equipment and components (for example, resins, matrices and cassettes) are used for purification purposes. QRM principles should be applied to devise the control strategy regarding such equipment and associated components when used in campaign manufacture and in multi-product facilities. The reuse of components at different stages of processing of one product is discouraged but, if performed, should be validated. Acceptance criteria, operating conditions, regeneration methods, lifespan and sanitization or sterilization methods, cleaning process, and hold time between the use of reused components should be defined and validated. The reuse of components for different products is not acceptable.

12.8 Where adverse donor (human or animal) health information becomes available after procurement and/or processing, and this information relates to product quality, then appropriate measures should be taken – including product recall, if applicable.
12.9 Antibiotics may be used during the early stages of production to help prevent inadvertent microbial contamination or to reduce the bioburden of living tissues and cells. In this case, the use of antibiotics should be well justified, and they should be cleared from the manufacturing process at the stage specified in the marketing authorization. Acceptable residual levels should be defined and validated. Penicillin and other beta-lactam antibiotics should not be used at any stage of the process.

12.10 A procedure should be in place to address equipment and/or accessories failure (such as air vent filter failure) which should include a product impact review. If such failures are discovered following batch release the NRA should be notified and the need for a batch recall should be considered.

13. Campaign production

13.1 The decision to use a facility or filling line for campaign manufacture should be justified in a documented manner and should be based on a systematic risk approach for each product (or strain) taking into account the containment requirements and the risk of cross-contamination to the next product. Campaign changeover procedures, including sensitive techniques used for the determination of residues, should be validated and proper cleaning acceptance criteria should be defined on a toxicology basis of product residues from the last campaign, as applicable. Equipment assigned to continued production or to campaign production of successive batches of the same intermediate product should be cleaned at appropriate validated intervals to prevent build-up and carry-over of contaminants (such as product degradants or objectionable levels of microorganisms).

13.2 For downstream operations of certain products (for example, pertussis or diphtheria vaccines) campaign production may be acceptable if well justified. For finishing operations (formulation and filling) the need for dedicated facilities or the use of campaigns in the same facility will depend on the specific characteristics of the biological product, on the characteristics of the other products (including any non-biological products), on the filling technologies used (such as single-use closed systems) and on local NRA regulations. Labelling and packaging operations can be carried out in a multi-product facility.

13.3 Campaign changeover involves intensive decontamination/sterilization (if required) and cleaning of the equipment and manufacturing area. Decontamination/sterilization (if required) and cleaning should include all equipment and accessories used during production, as well as the facility itself. The following recommendations should be considered:
- waste should be removed from the manufacturing area or sent to the bio-waste system in a safe manner;
- materials should be transferred by a validated procedure;
- the Quality Unit should confirm area clearance by inspection, and review the campaign changeover data (including monitoring results) prior to releasing the area for the next product.

13.4 When required, the corresponding diluent for the product can be filled in the same facility in line with the defined campaign production strategy for finished product.

13.5 When campaign-based manufacturing is considered, the facility layout and the design of the premises and equipment should permit effective cleaning and decontamination/sterilization (if required) based on QRM principles and validated procedures following the production campaign. In addition, consideration may need to be given at the design stage of facility layout to the possible need for fumigation.

14. Labelling

14.1 The information provided on the inner label (also called the container label) and on the outer label (on the packaging) should be readable and legible, and the content approved by the NRA.

14.2 Minimal key information should be printed on the inner label, and additional information should be provided on the outer label (for example, carton) and/or product leaflet.

14.3 The suitability of labels for low and ultra-low storage temperatures should be verified, if applicable. The label should remain properly attached to the container under different storage conditions during the shelf-life of the product. The label and its adhesive should have no adverse effect on the quality of the product caused by leaching, migration and/or other means.

15. Validation

15.1 Biological processes, handling of live materials and using campaign-based production, if applicable, are the major aspects of biological product manufacturing which require process and cleaning validation. The validation of such processes – given the typical variability of biological products, the possible use of harmful and toxic materials and the need for inactivation processes – plays an important role in demonstrating production consistency and in proving that the critical process parameters
and product attributes are controlled. Where available, WHO guidance documents should be consulted on the validation of specific manufacturing methods (for example, virus removal or inactivation (21)).

15.2 A QRM approach should be used to determine the scope and extent of validation.

15.3 All critical biological processes (including inoculation, multiplication, fermentation, cell disruption, inactivation, purification, virus removal, removal of toxic and harmful additives, filtration, formulation and aseptic filling) are subject, as applicable, to process validation. Manufacturing control parameters to be validated may include specific addition sequences, mixing speeds, time and temperature controls, limits of light exposure and containment.

15.4 After initial process validation studies have been finalized and routine production has begun, critical processes should be subject to monitoring and trending with the objective of assuring consistency and detecting any unexpected variability. The monitoring strategy should be defined, taking into consideration factors such as the inherent variability, complexity of quality attributes and heterogeneity of biological products. A system or systems for detecting unplanned departures from the process as designed should be in place to ensure that the process remains in a state of control. Collection and evaluation of information and data on the performance of the process will allow for detection of undesired process variability and will determine whether action should be taken to prevent, anticipate and/or correct problems so that the process remains under control.

15.5 Cleaning validation should be performed in order to confirm the effectiveness of cleaning procedures designed to remove biological substances, growth media, process reagents, cleaning agents, inactivation agents and so on. Careful consideration should be given to cleaning validation when campaign-based production is practised.

15.6 Critical processes for inactivation or elimination of potentially harmful microorganisms of Biosafety Risk Group 2 or above, including genetically modified ones, are subject to validation.

15.7 Process revalidation may be triggered by a process change as part of the change-control system. In addition, because of the variability of processes, products and methods, process revalidation may be conducted at predetermined regular intervals according to risk considerations. A detailed review of all changes, trends and deviations occurring within
a defined time period – for example, 1 year, based on the regular product quality review (PQR) – may indicate a need for process revalidation.

15.8 The integrity and specified hold times of containers used to store intermediate products should be validated unless such intermediate products are freshly prepared and used immediately.

16. Quality control

16.1 As part of quality control sampling and testing procedures for biological materials and products, special consideration should be given to the nature of the materials being sampled (for example, the need to avoid contamination, ensure biocontainment and/or cold chain requirements) in order to ensure that the testing carried out is representative.

16.2 Samples for post-release use typically fall into one of two categories – reference samples or retention samples – for the purposes of analytical testing and identification respectively. For finished products the reference and retention samples will in many instances be presented identically as fully packaged units. In such circumstances, reference and retention samples may be regarded as interchangeable.

Reference samples of biological starting materials should be retained under the recommended storage conditions for at least 1 year beyond the expiry date of the corresponding finished product. Reference samples of other starting materials (other than solvents, gases and water) as well as intermediates for which critical parameters cannot be tested in the final product should be retained for at least 2 years after the release of the product if their stability allows for this storage period. Certain starting materials such as components of growth media need not necessarily be retained.

Retention samples of a finished product should be stored in their final packaging at the recommended storage conditions for at least 1 year after the expiry date.

16.3 For cell-based products, microbiological tests (for example, sterility tests or purity checks) should be conducted on cultures of cells or cell banks free of antibiotics and other inhibitory substances in order to provide evidence of the absence of bacterial and fungal contamination, and to be able to detect fastidious organisms where appropriate. Where antibiotics are used, they should be removed by filtration at the time of testing.

16.4 The traceability, proper use and storage of reference standards should be ensured, defined and recorded. The stability of reference standards
should be monitored, and their performance trended. The WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards (30) should be followed.

16.5 All stability studies – including real-time/real-condition stability, accelerated stability and stress testing – should be carried out according to relevant WHO and other guidelines (31) or other recognized documents. Trend analysis of the test results from the stability monitoring programme should assure the early detection of any process or assay drift, and this information should be part of the PQR of biological products.

16.6 For products where ongoing stability monitoring would normally require testing using animals, and no appropriate alternative or validated techniques are available, the frequency of testing may take into account a risk-based approach. The principle of bracketing and matrix designs may be applied if scientifically justified in the stability protocol.

16.7 All analytical methods used in the quality control and in-process control of biological products should be well characterized, validated and documented to a satisfactory standard in order to yield reliable results. The fundamental parameters of this validation include linearity, accuracy, precision, selectivity/specificity, sensitivity and reproducibility (32–35).

16.8 For test methods described in relevant pharmacopoeial monographs, qualification of the laboratory test equipment and personnel should be performed. In addition, repeat precision and comparability precision should be shown in the case of animal tests. Repeatability and reproducibility should also be demonstrated by reviewing retrospective test data.

In addition to the common parameters typically used for validating assays (such as accuracy and precision) additional measurements (for example, of the performance of references, critical reagents and/or cell lines) should be considered during the validation of bioassays based on the biological nature of the assay and reagents used.

17. Documentation (batch processing records)

17.1 In general, the processing records of regular production batches should provide a complete account of the manufacturing activities of each batch of biological product showing that it has been produced, tested and dispensed into containers in accordance with the approved procedures.

In the case of vaccines, a batch processing record and a summary protocol should be prepared for each batch for the purpose of lot release
by the NRA. The information included in the summary protocol should follow the WHO Guidelines for independent lot release of vaccines by regulatory authorities (36). The summary protocol and all associated records should be of a type approved by the NRA.

17.2 Manufacturing batch records should be retained for at least 1 year after the expiry date of the batch of the biological product and should be readily retrievable for inspection by the NRA. It has been found that documents retained for longer periods may provide useful information related to AEFI and other investigations.

17.3 Starting materials may require additional documentation on source, origin, supply chain, method of manufacture and controls applied in order to ensure an appropriate level of control, including of microbiological quality if applicable.

17.4 Some product types may require a specific definition of what materials constitute a batch – particularly somatic cells in the context of ATMPs. For autologous and donor-matched situations, the manufactured product should be viewed as a batch.

18. Use of animals

18.1 A wide range of animals is used for the manufacture or quality control of biological products. Special considerations are required when animal facilities are present at a manufacturing site.

18.2 The presence of live animals in the production area should be avoided unless otherwise justified. Embryonated eggs are allowed in the production area, if applicable. If the extraction of tissues or organs from animals is required then particular care should be taken to prevent contamination of the production area (for example, appropriate disinfection procedures should be undertaken).

18.3 Areas used for performing tests involving animals or microorganisms should be well separated from premises used for the manufacturing of products and should have completely separate ventilation systems and separate staff. The separation of different animal species before and during testing should be considered, as should the necessary animal acclimatization process, as part of the test requirements.

18.4 In addition to monitoring compliance with TSE regulations (24) other adventitious agents that are of concern (including those causing zoonotic diseases and diseases in source animals) should also be monitored and
recorded in line with specialist advice on establishing such programmes. Instances of ill health occurring in the source/donor animals should be investigated with respect to their suitability, and the suitability of in-contact animals, for continued use (for example, in manufacture, as sources of starting materials, and for quality control and safety testing). Decisions should be documented.

18.5 A look-back procedure should be in place in relation to the decision-making process used to evaluate the continued suitability of the biological active substance or finished product in which animal-sourced starting materials have been used or incorporated. This decision-making process may include the retesting of reference samples from previous collections from the same donor animal (where applicable) to establish the last negative donation. The withdrawal period of therapeutic agents used to treat source/donor animals should be documented and should be taken into account when considering the removal of those animals from the programme for defined periods.

18.6 Particular care should be taken to prevent and monitor infections in source/donor animals. Measures taken should cover aspects such as sourcing, facilities, husbandry, biosafety procedures, testing regimes, control of bedding and feed materials, 100% fresh air supply, appropriate design of the HVAC system, water supply and appropriate temperature and humidity conditions for the species being handled. This is of special relevance to SPF animals where pharmacopoeial monograph requirements should be met. Housing and health monitoring should also be defined for other categories of animals (for example, healthy flocks or herds).

18.7 For products manufactured from transgenic animals, traceability should be maintained in the creation of such animals from the source animals. Note should be taken of national requirements for animal quarters, care and quarantine.

18.8 For different animal species and lines, key criteria should be defined, monitored and recorded. These may include the age, sex, weight and health status of the animals.

18.9 Animals, biological agents and tests carried out should be appropriately identified to prevent any risk of mix-up and to control all identified hazards.

18.10 The facility layout should ensure a unidirectional and segregated flow of healthy animals, inoculated animals and waste-decontamination areas. Personnel and visitors should also follow a defined flow in order to avoid cross-contamination.
19. Authors and acknowledgements

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20. References


Annex 3

Regulatory assessment of approved rDNA-derived biotherapeutics

Addendum to Annex 4 of WHO Technical Report Series, No. 987

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Guidance documents published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products.
Abbreviations

ALIFAR  Asociación Latinoamericana de Industrias Farmacéuticas
DCVMN  Developing Countries Vaccine Manufacturers Network
DNA   deoxyribonucleic acid
EGA    European Generic Medicines Association
ICDRA  International Conference of Drug Regulatory Authorities
IFPMA  International Federation of Pharmaceutical Manufacturers & Associations
IGPA   International Generic Pharmaceutical Alliance
NRA    national regulatory authority
rDNA   recombinant DNA
RBP    reference biotherapeutic product
SBP    similar biotherapeutic product
1. Introduction

This WHO guidance document considers the regulatory assessment needed to address situations where, for various reasons, biotherapeutic protein products prepared by recombinant deoxyribonucleic acid (DNA) technology (rDNA-derived biotherapeutics) were licensed with data packages that do not follow current international regulatory standards for these biologicals. This includes, for example, biotherapeutic products licensed via a generic pathway or with limited analytical, nonclinical and/or clinical data (1, 2). At its 2010 meeting in Singapore (3) the International Conference of Drug Regulatory Authorities (ICDRA) discussed such situations and requested WHO assistance in developing approaches for evaluating these already-licensed products in accordance with current WHO guidelines. In May 2014 the Sixty-seventh World Health Assembly adopted two relevant resolutions: one on promoting access to biotherapeutic products and ensuring their quality, safety and efficacy (4) and the other on regulatory systems strengthening (5) in which WHO was requested to provide guidance, especially on dealing with increasingly complex biological products.

Although primarily addressing rDNA-derived biotherapeutic protein products, some aspects of this document may also be relevant to other biotherapeutics.

2. Regulatory expectations for rDNA-derived biotherapeutics, including similar biotherapeutic products

The regulatory expectations for rDNA-derived biotherapeutics can be found in the relevant WHO Guidelines adopted by the 2013 WHO Expert Committee on Biological Standardization (6). Following extensive consultation at the global level since 2004, WHO Guidelines on the evaluation of similar biotherapeutic products (SBPs) were adopted by the Committee in 2009 (7). These latter WHO Guidelines emphasize the need for a head-to-head demonstration of the “similarity” of such products to reference biotherapeutic products (RBPs) of assured quality, safety and efficacy that have been licensed on the basis of a full licensing dossier. A head-to-head comparability exercise between a candidate SBP and an RBP is essential to justify a reduced nonclinical and clinical package for licensing (7). Studies should be designed to demonstrate comparability and to detect any potential difference in quality, nonclinical and clinical attributes between the SBP and RBP rather than simply to confirm the safety and efficacy of the two products. It should be ensured that any differences that are detected have no clinically meaningful impact on product performance.
If a head-to-head comparison of the SBP with the RBP as outlined in the WHO Guidelines for SBPs (7) is not performed throughout the development process then the final product should not be referred to as an SBP (8, 9). SBPs are not “generic medicines” and the approval process used for small-molecule generics is not applicable.

3. Review of products on the market

Problems have been identified in some countries where, for various reasons, biotherapeutic products were licensed using data which no longer meet current WHO regulatory expectations – such as biotherapeutic products licensed as generics or as small-molecule drugs. In many cases pharmacovigilance systems in such countries are weak or even nonexistent, with the result that little is known about the safety and efficacy of individual products. In addition, the terminology used for such products is confusing and their traceability poor (10, 11). In some countries, the coexistence on the market of these products and SBPs, as well as rDNA-derived biotherapeutics licensed with full data packages, is a matter of concern. This was the situation for both erythropoietin (12) and heparin (13). Some updating of national regulations has occurred to take account of the recognized difficulties and changes made in international regulatory expectations (14–17). Special considerations apply to the production and control of biological medicines, including biotherapeutics, which do not apply to chemical drugs. This is because of the biological nature of the starting materials, the manufacturing processes and the test methods needed to characterize batches of the product – as well as the highly complex molecular structure of products themselves. Nonclinical and clinical evaluations are key components of the regulatory assessment of all biotherapeutics. Products already approved under the pre-existing regulations will need to be reassessed to ensure that they meet the new requirements.

National regulatory authorities (NRAs) should undertake a stepwise regulatory review of all biotherapeutic products already authorized for marketing, as follows:

1. First, NRAs should identify products that have been licensed with data which do not meet current WHO regulatory expectations.
2. Second, an assessment of identified products and gaps, based on the product-specific considerations listed below in section 4, should be carried out in order to decide upon the appropriate action needed to remedy the situation, and to determine the timelines for implementing this action. This will inevitably involve a risk–benefit assessment of the situation.
3. Third, manufacturers should submit a plan of action for dealing with the problem to the NRA within a defined – but short – period of time. The plan of action should consist of an analysis of available and missing data in accordance with WHO Guidelines (6, 7), as well as a description of measures (which may include interim assessments) and proposed timelines needed to address the identified gaps.

4. Fourth, NRAs should evaluate the plan of action proposed by the manufacturer and reach agreement with the manufacturer on the next steps for generating missing data and for their (possibly stepwise) submission to the NRA.

5. Fifth, NRAs should assess the submitted data (for example, quality/manufacturing, nonclinical and clinical data as needed) using a stepwise approach – possibly in several separate packages at different times – and decide upon the appropriate regulatory action to take based upon the assessment outcome.

The timeline for completing the overall review exercise will depend upon the time needed to generate and provide the missing information, taking into consideration the product-specific points outlined below in section 4. For example, in 2009 one NRA clarified the “appropriate regulatory pathway” for dealing with changes in the regulatory oversight of low molecular weight heparins\(^1\) to reflect the fact that in future they would be regulated in that country as biologicals and not as small-molecule pharmaceuticals (16). In addition, it was announced that any biosimilar heparin submissions should follow the regulatory framework for biosimilars and not the generic pathway. A transition period of 12 months was set to allow manufacturers to update their files to reflect the data required for biologicals. Manufacturers were also required to report on how much of their licensed product was sold in the country per year following the official start date of the revised regulatory approach.

Similar transitional provisions have been made by other NRAs when updating the regulations for biotherapeutics, including biosimilars (15, 17).

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\(^1\) Low molecular weight heparin is not an rDNA-derived biotherapeutic product but is highlighted here as an example of a reviewed product on the market. It is not considered to be a biological product in some countries.
4. Points to consider in a stepwise regulatory assessment

A particular licensed product should be allowed to remain on the market during the review process unless specific causes or events lead the NRA to make its own judgment to suspend market availability of the product during the review process. Consideration should be given to the following points when deciding upon the appropriate regulatory actions:

- NRAs should consider: (a) the number of products on the market which have been licensed without adequate quality, nonclinical and/or clinical data; and (b) the availability of alternative therapeutics on that market licensed locally with an adequate data package and/or licensed by an experienced NRA that meet the standards of the relevant WHO guidelines (see next bullet point).

- It is important to find out if the product in question is manufactured and licensed in a country with a jurisdiction which has, and follows, well-established regulatory frameworks, including as appropriate all the principles set out in the relevant WHO Guidelines for rDNA-derived biotherapeutics (6) and in WHO Guidelines for SBPs (7). Account should also be taken of whether the jurisdiction concerned has considerable experience in the evaluation of biotherapeutic products (including SBPs) and post-marketing surveillance activities. If a product is manufactured and/or licensed in a country with considerable experience in these areas then this provides some degree of confidence regarding product quality, safety and efficacy. In addition, it is important to ascertain whether the actual product authorized in the country with limited regulatory experience is comparable – with respect to manufacturing process and controls, recent good manufacturing practices inspection and labelling – to the product licensed, supplied and used in the manufacturing country with the more experienced jurisdiction. It is also important to determine whether registration of the product in question has been rejected, cancelled or suspended by other experienced NRAs.

- It is also important to know the extent to which the registration dossier of the biotherapeutic product meets the recommendations set out in the above WHO Guidelines (6, 7). Attention should be paid to any key differences between national requirements and the WHO Guidelines – such as the lack of a head-to-head comparability exercise for an SBP. The NRA should provide manufacturers with a critical dataset for the re-registration of such products. Changes in regulatory requirements may be needed, as well as amendments to
the legal framework of the country concerned, to enable such new requirements to be implemented.

- The necessity and extent of use of a biotherapeutic product along with the availability of alternative products (if any) should be ascertained. This would include, for example, assessing whether the product was essential for treating certain patients and what the clinical outcomes would be if the product was taken off the market. This assessment should cover: (a) the disease that is being treated; (b) whether the condition is life threatening; (c) the consequences of treating or not treating, or of stopping treatment in patients already using the product; (d) the risk of switching between therapeutic alternatives; (e) the likelihood (and potential consequences, if any) of supply problems on clinical outcomes should the product be taken off the market; and (f) the type of patient population (for example, paediatric, adult or older persons).

- The seriousness of a potential lack of efficacy should be considered, as should possible safety issues (including higher efficacy) that may result from the continued use of the product under review. This should include an assessment of the severity of the potential impact on a patient of an immunogenic effect arising from the use of the product and an assessment of any adverse effects. Such effects might include cross-reactivity with native proteins caused by biotherapeutic products – such as pure red cell aplasia caused by erythropoietin (1).

- Consideration should also be given to the ability of the pharmacovigilance system in the country to detect and monitor any potential adverse reactions and/or efficacy problems (such as reduced clinical effectiveness) associated with the biotherapeutic product.

- Criteria for the evaluation of functional pharmacovigilance systems have been developed by WHO (18). Given the poor pharmacovigilance systems in many countries, as well as terminology difficulties, it may not be possible to obtain sufficient data to demonstrate that a particular product was the cause of an adverse reaction or that patients may be at risk from the use of products that are clinically untested or were tested in inadequately designed studies. Traceability is a key element in monitoring the safety and efficacy of biologicals as it enables pharmacovigilance measures to be put in place.

- The expertise and capacity of regulators responsible for licensing biotherapeutic products are critically important factors in the
appropriate evaluation of these products. Collaboration between NRAs, including work-sharing agreements and joint reviews with other NRAs, should also be explored (*for example, see 2, 19, 20*).

- Consideration should be given to ensuring transparency with respect to informing health-care professionals, pharmacists and patients of the review process and its timelines. This could be done through website posting (16), via a symbol and some text in the product information or any other means the NRA is allowed to use, highlighting the need to align the licensing process with current international expectations. This could also provide an opportunity to request users to report any safety and/or efficacy issues.

### 5. Regulatory actions

On the basis of the outcomes of the regulatory assessment, the NRA should decide upon the appropriate actions to be taken. The decisions and actions of NRAs may differ depending upon the assessments made according the points listed above in section 4, which will be jurisdiction specific. In a stepwise approach, product supply would not be compromised and authorization might be regularized after the defined time period during which the product would have undergone further regulatory evaluation, and on condition that it was shown to have an acceptable risk–benefit profile.

Capacity-building will be needed where resources and expertise are considered inadequate. Where the number and level of experience of staff available to undertake an overall review are limited, consideration could be given to mentoring or to work-sharing arrangement amongst NRAs. In the case of mentoring, support could be provided through WHO from an experienced authority that uses well-established processes that accord with relevant WHO guidelines. In addition, the sharing of information between NRAs regarding the basis for regulatory decisions on biotherapeutic products (including SBPs) and the availability of publically available evaluation reports are considered important sources of support for regulatory authorities that are less experienced in dealing with these highly complex products, and may accelerate product assessment. Communicating the details of what information was reviewed and how it was incorporated into decision-making is also important for prescribers, patients and other stakeholders, and can help promote confidence in biotherapeutic products. The summary basis of decision documents produced by some regulatory agencies, such as Health Canada, the European Medicines Agency and the United States Food and Drug Administration, are examples of informative documents.
The stepwise regulatory assessment approach outlined in this document is flexible and designed to increase the accessibility of biotherapeutic products of assured quality, safety and efficacy, as called for in the relevant 2014 World Health Assembly resolutions (4, 5).

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7. References


Annex 4

Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines

Replacement of Annex 1 of WHO Technical Report Series, No. 962

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Recommendations published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these WHO Recommendations are made only on condition that such modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. The parts of each section printed in small type are comments or examples intended to provide additional guidance to manufacturers and NRAs.
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CIN</td>
<td>cervical intraepithelial neoplasia</td>
</tr>
<tr>
<td>CIN2–3</td>
<td>CIN grades 2 or 3</td>
</tr>
<tr>
<td>CIN2+</td>
<td>cervical intraepithelial neoplasia grade 2 or worse</td>
</tr>
<tr>
<td>cLIA</td>
<td>competitive Luminex immunoassay</td>
</tr>
<tr>
<td>CMI</td>
<td>cell-mediated immunity</td>
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<tr>
<td>DCVMN</td>
<td>Developing Countries Vaccine Manufacturers Network</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EIA</td>
<td>enzyme immunoassay</td>
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<tr>
<td>GCP</td>
<td>good clinical practice</td>
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<tr>
<td>GMC</td>
<td>geometric mean concentration</td>
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<tr>
<td>GMT</td>
<td>geometric mean titre</td>
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<tr>
<td>HPV</td>
<td>human papillomavirus</td>
</tr>
<tr>
<td>IARC</td>
<td>WHO International Agency for Research on Cancer</td>
</tr>
<tr>
<td>ICP</td>
<td>immune correlate of protection</td>
</tr>
<tr>
<td>IFPMA</td>
<td>International Federation of Pharmaceutical Manufacturers &amp; Associations</td>
</tr>
<tr>
<td>IU</td>
<td>International Unit(s)</td>
</tr>
<tr>
<td>LAL</td>
<td>limulus amebocyte lysate</td>
</tr>
<tr>
<td>LLOD</td>
<td>lower limit of detection</td>
</tr>
<tr>
<td>LLOQ</td>
<td>lower limit of quantification</td>
</tr>
<tr>
<td>MCB</td>
<td>master cell bank</td>
</tr>
<tr>
<td>MOI</td>
<td>multiplicity of infection</td>
</tr>
<tr>
<td>MPL</td>
<td>monophosphoryl lipid A</td>
</tr>
<tr>
<td>NAT</td>
<td>nucleic acid amplification technique</td>
</tr>
<tr>
<td>NCL</td>
<td>national control laboratory</td>
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<tr>
<td>NRA</td>
<td>national regulatory authority</td>
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<tr>
<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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</tbody>
</table>
PDL  population doubling level
RBC  red blood cell
RRP  recurrent respiratory papillomatosis
SAGE  WHO Strategic Advisory Group of Experts
SDS-PAGE  sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC–HPLC  size-exclusion chromatography – high-performance liquid chromatography
spp.  species
TEM  transmission electron microscopy
VLP  virus-like particle
WCB  working cell bank
Introduction

WHO Guidelines to assure the quality, safety and efficacy of recombinant human papillomavirus (HPV) virus-like particle (VLP) vaccines were first adopted by the WHO Expert Committee on Biological Standardization in 2006 (1) and were based largely on experience gained from clinical trials undertaken on the first two licensed HPV vaccines.

The factors that have prompted this revision include the substantial amount of data accumulated during vaccine implementation, the development of prophylactic vaccines with extended valency and the use of other production methods. In addition, the increasing availability and routine use of HPV VLP vaccines composed of L1 capsid protein and containing at least types 16 and 18 have important implications for trial designs and end-points for clinical evaluation of new prophylactic HPV vaccines.

A series of meetings was convened by WHO to review the scientific evidence needed to initiate and inform the revision process. These meetings were attended by experts from around the world involved in the research, manufacture, licensing/authorization, control-testing and release of HPV vaccines. Participants were drawn from academia, national regulatory authorities (NRAs), national control laboratories (NCLs) and industry, and included representatives of the WHO Global HPV LabNet – an initiative that worked towards the international standardization of HPV testing during 2006–2011. These experts reviewed new HPV vaccines under development, and the scientific basis and evidence for accepting alternative end-points for evaluating the clinical efficacy of candidate HPV vaccines. The first meeting held was in February 2013 and considered issues relating to the development and evaluation of clinical end-points for trials of new HPV vaccines and other issues to be addressed in the proposed revision. At a meeting held at the WHO International Agency for Research on Cancer (IARC), Lyon, France in September 2013 a Working Group discussed whether it might be appropriate to consider using a virological end-point – rather than a disease end-point such as cervical intraepithelial neoplasia (CIN) grade 2 or worse (CIN2+) – as the primary end-point for future clinical efficacy trials, and the circumstances under which immunobridging trials might be sufficient for licensure (2, 3). A third meeting held at WHO headquarters in November 2013 reviewed and discussed the outcomes of the IARC scientific meeting on appropriate clinical end-points, reviewed vaccines currently in the development pipeline, and assessed regulatory and laboratory needs for licensing the vaccines (4).

Major issues addressed in these resulting WHO Recommendations include updates of:

- terminology;
- general considerations and other sections to reflect the up-to-date development of HPV vaccines;
the section on international standards and reference preparations;
- Part A, in order to include bacteria as a cell substrate;
- Part B, in order to include a new subsection highlighting tests needed for nonclinical evaluation, and to elaborate regulatory requirements for both proof-of-concept pharmacology studies and toxicological testing;
- Part C, in order to reflect appropriate and feasible end-points in future trials;
- appendices.

Additional changes have also been made to bring the document into line with other WHO Recommendations, Guidelines and guidance documents published since the 2006 adoption of the WHO Guidelines on recombinant HPV VLP vaccines (1).

Scope

These WHO Recommendations provide guidance to NRAs and manufacturers on the manufacturing process, and on nonclinical and clinical aspects, of recombinant HPV VLP vaccines to assure their quality, safety and efficacy.

The scope of the present document encompasses recombinant HPV VLP vaccines for prophylactic use which contain the L1 capsid protein of one or more HPV types.

The document does not cover vaccines targeted to L2 capsid proteins as antigens, as appropriate serological assays have not yet been standardized and clinical vaccine trials have not started. Non-VLP vaccines (for example, other forms of subunit vaccines, vectored vaccines and L1 capsomers) and investigational therapeutic HPV vaccines, which are at an early stage of development, are also not included. However, some aspects discussed below may be relevant and may be taken into consideration during vaccine development.

This document should be read in conjunction with other relevant WHO guidance such as that on nonclinical (5) and clinical (6) evaluation of vaccines. Other WHO guidance, such as that on the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7) and nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (8), should also be considered.

General considerations

HPV is not a single virus; rather, it includes a group of closely related small, non-enveloped deoxyribonucleic acid (DNA) viruses in the Papillomaviridae
family. The circular, double-stranded viral genome is approximately 8 kb in length. The genome encodes for six early protein-coding sequences responsible for virus replication and two late proteins (L1 and L2) which are the viral structural capsid proteins. L1 is the major structural protein. L1 proteins associate to form pentameric structures called capsomers (9). Mature virus particles comprise 72 capsomers arranged in icosahedral symmetry. The minor capsid protein, L2, is present in as many as 72 molecules per mature virus particle (10). L2 is not required for particle formation, but plays a role in encapsidating the genome. HPV infection, replication and particle maturation occur in the stratified squamous epithelia of skin and mucous membranes (11).

Over 190 different types of HPV have been identified and molecularly characterized (12). These HPVs cause a variety of epithelial diseases in humans, ranging from benign warts to cancers (including of the cervix, vagina, vulva, penis, anus and oropharynx). HPV types associated with the development of cervical cancer are labelled as high risk for oncogenicity. Other HPV types, such as types 6 and 11 which are associated with genital warts, are considered as low risk for oncogenicity.

The majority of HPV infections with both high-risk and low-risk types are asymptomatic, self-limiting and resolve spontaneously due to the host immune response. However, in rare instances HPV infection persists. If persistent infection with high-risk types is not detected and treated then progression to invasive carcinoma may occur at the site of infection. The interval between acquisition of HPV infection and malignant (invasive) progression is generally at least 10 years. As high-risk HPV types are detected in virtually all cervical cancers, it is scientifically accepted that the persistent viral infection is necessary though not sufficient for the development of cancer (13, 14). The basis for progression to invasive carcinoma is not well understood. Environmental and physiological cofactors such as high parity, hormonal contraceptives and smoking may increase the risk for cancer development in people with persistent infection (15–17). Nevertheless, individuals without identified risk factors who have persistent infection can also develop cervical cancer.

IARC currently defines 12 high-risk HPV types that are associated with cancers in humans – namely HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (Group 1) – and an additional type as probably carcinogenic – namely HPV type 68 (Group 2A) (14). HPV type 66 was formerly classified as oncogenic but recent findings have significantly weakened the evidence for this. Additional data on rare HPV types currently considered possibly oncogenic to

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1 The terms oncogenic/oncogenicity and carcinogenic/carcinogenicity are both used in the scientific literature on HPVs. In this document the terms oncogenic/oncogenicity are used.
humans (Group 2B) suggest that some, but not all, could be upgraded (18). The distribution and prevalence of the above HPV types in patients with cancer are generally consistent around the world. Two of the high-risk HPV types (16 and 18) account for approximately 70% of all cervical cancers globally (14). Most anal cancers are also associated with persistent HPV infection, with HPV type 16 representing an even higher fraction (90%) of HPV-positive cancers of the anus than is the case for cervical cancer (approximately 50%). In addition, these high-risk HPV types are associated with a significant fraction of cancers of the vagina, vulva, penis and oropharynx. The incidence of cervical cancer is substantially higher than that of all other HPV-related cancers; cervical cancer is the second most common cancer among women aged 15–44 years.

Low-risk HPV types cause genital warts, recurrent respiratory papillomatosis (RRP) and low-grade cervical dysplasia. Genital warts affect both males and females. Data on the worldwide burden of genital warts are not available, but in developed countries the epidemiology is similar to other sexually transmitted infections, peaking in young ages (15–24 years) (19). While not malignant, these lesions are associated with physical and psychological morbidity and may be difficult to treat. RRP is a devastating, although rare, disease that manifests as recurrent, rapidly growing benign laryngeal tumours that require frequent excision to prevent airway obstruction. HPV types 6 and 11 are responsible for over 90% of genital warts and RRP cases, and for 9–12% of low-grade cervical dysplastic lesions.

Identification of a viral agent such as HPV as a major cause of diseases implies that prophylactic vaccines or interventions against the viral agent should prevent the disease(s) it causes. Initial studies in animal models showed that inoculation with species-specific papillomaviruses induced an immune response that conferred protection against homologous virus challenge. However, native papillomaviruses are not good substrates for vaccine development as they cannot be grown in standard cell culture. Subsequent studies showed that L1 protein produced in heterologous expression systems, such as yeast or insect cells, self-assembles into VLPs that are morphologically similar to authentic HPV virions but contain no viral DNA. In animal studies, VLPs were shown to protect against experimental infection with a high dose of homologous virus (20, 21). HPV VLPs are highly immunogenic in mice and rabbits, and the resulting antibodies have been shown to be neutralizing and type-restricted when tested in a pseudovirion neutralization assay. In animal studies, immunization with denatured VLPs did not produce neutralizing antibodies and did not give protection against experimental virus challenge, indicating that neutralizing epitopes are conformation-dependent.

Protection in animals has been demonstrated through passive transfer of antibodies in serum (20–22). Neutralizing antibodies are believed to be the primary mediator of this protection. Enhanced regression of established HPV
lesions, which requires cell-mediated immunity (CMI), was not observed after VLP vaccination. Therefore, it seems unlikely that CMI is substantially involved as a direct effector mechanism of protection (23). The specific assays that have been developed to evaluate the humoral immune response to HPV include VLP-based enzyme immunoassay (EIA), competitive immunoassay with labelled neutralizing monoclonal antibodies and pseudovirion-based neutralization assays (24).

The revision of the former WHO Guidelines to produce these WHO Recommendations has been driven by the experience acquired in using the first two licensed HPV L1 VLP vaccines in many countries, and by the development of an extended version of one of these vaccines to include additional HPV types. These vaccines are made up of recombinant protein L1 VLPs and contain adjuvant to enhance their immunogenicity. The vaccines are delivered via intramuscular injection. The products differ in the types of HPV L1 proteins included as antigens, substrates used for production, adjuvant properties and final formulation. The three vaccines are:

- A bivalent vaccine comprising HPV types 16 and 18 VLPs self-assembled from L1 protein expressed and purified from insect cells infected with a recombinant baculovirus; the vaccine is formulated with a novel adjuvant (AS04) which contains aluminium hydroxide and monophosphoryl lipid A (MPL) with each dose delivering 20 µg of each VLP.

- A quadrivalent vaccine comprising HPV types 6, 11, 16 and 18 VLPs self-assembled from L1 protein expressed and purified from Saccharomyces cerevisiae (baker’s yeast) containing L1 expression plasmids; the vaccine is formulated with amorphous aluminium hydroxyphosphate sulfate adjuvant with each dose delivering 20 µg of HPV types 6 and 18 VLPs and 40 µg of HPV types 11 and 16 VLPs.

- An extended version of the quadrivalent vaccine that contains five additional oncogenic HPV types (types 31, 33, 45, 52 and 58); the vaccine is manufactured as for the quadrivalent vaccine and contains the same adjuvant.

The bivalent vaccine expressed from recombinant baculovirus in insect cells was the first vaccine to be developed in this host expression system. Other novel expression systems, such as Escherichia coli or Pichia yeast, may be introduced. Testing of novel cell substrates may have some unique requirements. Each VLP type in a multivalent vaccine should be produced and purified separately, and then mixed together for the final formulation. L1 protein in its native form is not glycosylated (25). The currently available vaccines do not utilize expression systems that glycosylate the VLP, and glycosylation does not
appear to be important. Other expression systems without post-translation glycosylation, such as the E. coli expression system, could also be used.

L1 VLPs may include other recombinant proteins (for example, L2) that serve as a stabilizer rather than as an antigen (26). Conformational epitopes of L1 VLPs are required for eliciting neutralizing antibody response in the host. Disassembly of the L1 VLPs into capsomers and reassembly of the L1 capsomers back into VLPs may contribute to purification of some types of HPV VLPs and lead to more-stable VLPs.

Purified L1 VLP preparations will need to be characterized biochemically and immunologically to determine L1 concentration, purity, post-translational modification and assembly state. L1 VLPs incorporate nucleic acids of their producer cells to varying degrees, and so the amount of residual host cellular DNA incorporated needs to be determined. Removal of host cellular DNA may be required if the amount exceeds the limits set by NRAs for the specific production system.

Regulatory approvals for the bivalent and quadrivalent vaccines were based on double-blind efficacy studies in females aged 16–26 years using unvaccinated control groups – namely groups that received placebo or another vaccine with no potential to protect against HPV. The primary end-points for these Phase III clinical trials were based on histological detection of precancerous epithelial changes attributable to specific HPV types as a surrogate for risk of progression to malignant lesions (CIN grades 2 or 3 (CIN2–3) and adenocarcinoma in situ). Secondary end-points included 6- or 12-month type-specific persistence of HPV detection. Primary efficacy was demonstrated in women who were not previously exposed to the vaccine types. Immunogenicity and safety were established in younger males and females (ages 9–15 years), and this information was used to bridge efficacy claims to this population. On the basis of demonstrated safety and efficacy against cervical cancer precursors, the WHO Strategic Advisory Group of Experts (SAGE) on Immunization recommended that the primary target population should be girls within the age range of 9 or 10 years through to 13 years – that is, before the age of initiation of sexual activity and exposure to HPV (11).

The initial product licences were for 3-dose schedules (0, 1 or 2 months and 6 months). Subsequently the European Medicines Agency approved: (a) a 2-dose schedule for the bivalent vaccine for females aged 9–14 years; and (b) a 2-dose schedule for the quadrivalent vaccine for females aged 9–13 years. For both the bivalent and quadrivalent HPV vaccines, SAGE recommended a 2-dose schedule with a 6-month interval between doses for females younger than 15 years. Those who are ≥ 15 years at the time of the second dose are also adequately covered by 2 doses (11).

The extended version of the quadrivalent vaccine includes five additional HPV types (31, 33, 45, 52 and 58). All nine HPV VLPs are prepared from L1
protein expressed and purified from *S. cerevisiae* containing L1 expression plasmids. The licensure of this 9-valent vaccine was based on demonstration of efficacy against the additional HPV types (using a composite histological end-point) and a comparison of immune responses to the four HPV types in the quadrivalent vaccine.

The currently available HPV vaccines are manufactured in single-dose or 2-dose vials or prefilled syringes without the addition of preservative. In future, the availability of multi-dose vaccine vials should facilitate the adoption of innovative vaccination strategies targeting pre-adolescents and adolescents in developing countries. If these vaccines do not contain preservative, their use should be time-restricted as is the case for reconstituted vaccines such as Bacillus Calmette-Guerin vaccine and measles-containing vaccines (27). If a preservative were to be added its effect on antigenicity and immunogenicity must be assessed to ensure that the preservative does not affect immune response. Thiomersal, an organo-mercury compound that was a widely used preservative in vaccines, was found to destroy the major neutralizing epitope of HPV type 16 L1 VLPs when added to the vaccine formulation (28).

Current vaccine formulations use intramuscular administration. Other routes of administration – such as nasal, cutaneous or oral – may be investigated to facilitate vaccination by avoiding the use of needles. It is crucial that nonclinical studies demonstrate immunogenicity and the production of neutralizing antibodies for any new products and any new routes of administration.

**Terminology**

The definitions given below apply to the terms as used in these WHO Recommendations. They may have different meanings in other contexts.

**Adjuvant:** a substance or a combination of substances used in conjunction with a vaccine antigen to enhance (for example, increase, accelerate, prolong and/or possibly target) the specific immune response to the vaccine antigen and the clinical effectiveness of the vaccine. This may also be called a mineral vehicle or immunostimulant.

**Adsorbed monovalent antigen bulk:** a batch of purified monovalent antigen bulk adsorbed on adjuvant. Different batches of adsorbed monovalent antigen bulks may be pooled before collection into a single vessel.

If a novel adjuvant is used that does not involve adsorption of the VLP to the adjuvant, the term “adjuvanted monovalent antigen bulk” may be used.

**Adventitious agents:** contaminating microorganisms that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses, that have been unintentionally introduced into the manufacturing process.
Cell bank: a collection of containers containing aliquots of a suspension of cells from a single pool of uniform composition, stored frozen under defined conditions (typically −60 °C or below for yeast or bacteria and in liquid nitrogen for insect or mammalian cell lines).

The individual containers (for example, ampoules or vials) should be representative of the pool of cells from which they are taken and should be frozen on the same day following the same procedure and using the same equipment and reagents.

Cell seed: a quantity of vials containing well-characterized cells derived from a single tissue or cell of human or animal origin, stored frozen in liquid nitrogen in aliquots of uniform composition, one or more of which are used for the production of a master cell bank.

Cell substrate: cells used to manufacture a biological product.

Comparator vaccine: a licensed vaccine with established efficacy or with traceability to a vaccine with established efficacy that is tested in parallel with a candidate vaccine and serves as an active control in nonclinical or clinical testing.

Composite end-points: combine two or more single end-points in one outcome to demonstrate overall treatment effects. Subjects who have experienced any of the events specified in the single end-points are considered to have experienced the composite end-point (29).

Control cell culture: cells amplified from the working cell bank and split from those intended for use in production immediately prior to intentional viral infection (for example, with baculovirus expression vector) and grown in parallel for the purpose of serving as test material.

Expression construct: defined as the expression vector containing the coding sequence of the recombinant protein (30).

Expression system: the host cell with its expression construct and the cell culture process that is capable of expressing protein encoded by the expression construct. Expression systems can be bacterial-based, baculovirus-insect-cell-based, mammalian-based or yeast-based.

Final bulk: the formulated vaccine present in a container from which the final containers are filled. The final bulk may be prepared from one or more adsorbed monovalent antigen bulks and may contain VLP antigens from one or multiple HPV types. If prepared from one or more monovalent antigen bulks, mixing should result in a uniform preparation to ensure that the final containers are homogenous.

Final lot: a collection of sealed final containers of finished vaccine that is homogeneous with respect to the risk of contamination during the filling process. All of the final containers must therefore have been filled from a single vessel of final bulk in one working session.
HPV L1 protein: the major structural capsid protein of human papillomavirus, of which 360 molecules are found in the native virion associated in 72 pentameric capsomers.

Immune correlate of protection (ICP): an immune response parameter of a type (for example, serum antibodies) and amount above which an individual is most probably protected. For many vaccines, functional antibodies above a threshold geometric mean titre (GMT) or geometric mean concentration (GMC) serve as an ICP.

Immunobridging studies: studies intended to support the extrapolation of efficacy from one formulation, population or dose regimen to another based on comparison of relevant immune responses.

Inoculum intermediate: a quantity of recombinant baculovirus of uniform composition, derived from the recombinant baculovirus working seed lot. The inoculum intermediate has a defined shelf-life. It is intended to be used to initiate the production of recombinant L1 proteins from insect cells.

L1 virus-like particle (VLP): a non-infectious, non-replicating, non-enveloped, icosahedral capsid particle that resembles native virions but does not contain viral DNA. The regular arrays of L1 pentameric capsomers retain conformational epitopes.

Master cell bank (MCB): a quantity of well-characterized cells of animal or other origin, derived from a cell seed at a specific population doubling level (PDL) or passage level, dispensed into multiple containers, cryopreserved and stored frozen under defined conditions (typically −60 °C or below for yeast or bacteria, and in liquid nitrogen for insect or mammalian cell lines). The MCB is prepared from a single homogeneously mixed pool of cells. In some cases, such as genetically engineered cells, this may be prepared from a selected cell clone established under defined conditions. The MCB is used to derive all working cell banks for the anticipated lifetime of the vaccine product.

Parental cells: cells that are manipulated to give rise to a cell substrate.

Manipulation may be simply the expansion of a primary cell culture to provide early passage cells, or a more complex activity such as developing a transfected clone. Both processes would provide a cell seed. The parental cells may refer to any stage prior to the preparation of the cell seed.

Production cell culture: a collection of cell cultures used for the production of HPV L1 antigen that have been prepared together from one or more containers of the working cell bank.

Purified monovalent antigen bulk: a batch of purified antigen of a single HPV type. Different batches of purified monovalent antigen bulks may be pooled into a single vessel.

Recombinant baculovirus master seed lot: a quantity of recombinant baculovirus of uniform composition derived from an original baculovirus
expression construct, processed at one time and passaged for a documented number of times.

**Recombinant baculovirus working seed lot:** a quantity of recombinant baculovirus of uniform composition derived from the recombinant baculovirus master seed lot by a limited number of passages. The recombinant baculovirus working seed lot may be used to prepare inoculum intermediates or alternatively to initiate the production of recombinant L1 proteins.

**Single antigen harvest:** a concentrated cell suspension or supernatant containing the intended HPV antigens of one virus type harvested from cell cultures prepared from a single production run.

**Single harvest pool:** a homogeneous pool of multiple single harvests of the intended HPV antigens of one virus type, collected into a single vessel prior to purification.

**Working cell bank (WCB):** a quantity of well-characterized cells of animal or other origin, derived from the MCB at a specific PDL or passage level, dispensed into multiple containers and stored under defined culture conditions (typically −60 °C or below for yeast or bacteria, and in liquid nitrogen for insect or mammalian cell lines). The WCB is prepared from a single homogeneously mixed pool of cells. One or more of the WCB containers are used for each production culture. Multiple WCBs may be made and used during the lifetime of the vaccine product.

**Part A. Manufacturing recommendations**

A.1 **Definitions and international reference materials**

A.1.1 **International name and proper name**

The international name should be “recombinant human papillomavirus virus-like particle vaccine” followed in parentheses by the type specificity and the name of the recombinant protein (for example, types 16 and 18 L1 proteins). The proper name should be equivalent to the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that meet the specifications elaborated below.

A.1.2 **Descriptive definition**

The recombinant HPV VLP vaccine is a sterile liquid vaccine preparation that contains purified VLPs composed of the recombinant major capsid proteins (L1) of one or more HPV types. The VLPs may be formulated with a suitable adjuvant. Such vaccines are for prophylactic use.
A.1.3 **International reference materials**

International Standards for HPV types 16 and 18 antibodies are available. These materials may be used in immunoassays utilizing VLP binding assays and pseudovirion neutralization tests of adequate sensitivity \((31, 32)\).

International Standards for HPV types 16 and 18 DNA are also available. These standards are suitable for the calibration of in-house or working standards for the amplification and detection of HPV types 16 and 18 DNA \((33)\).

The reference materials listed above are available from the National Institute for Biological Standards and Control, Potters Bar, the United Kingdom.

The WHO Catalogue of International Reference Preparations should be consulted for the latest list of appropriate WHO International Standards and reference materials \((34)\). The *Human papillomavirus laboratory manual* \((24)\) provides guidance on the preparation and use of secondary standards calibrated against International Standards for HPV DNA and antibodies.

International Standards and Reference Reagents for the control of HPV VLP vaccine antigen content and potency are not available. Therefore, product-specific reference preparations may be used.

A.2 **General manufacturing recommendations**

The general manufacturing requirements contained in WHO good manufacturing practices for pharmaceutical products: main principles \((35)\) and WHO good manufacturing practices for biological products \((36)\) should apply to the design, establishment, operation, control and maintenance of manufacturing facilities for recombinant HPV VLP vaccines, with the addition of the following:

- Production steps involving manipulations of recombinant HPV L1 VLP types should be conducted at a biosafety level consistent with the production of recombinant microorganisms.
- Quality control procedures should be in place to ensure segregation of different HPV L1 VLP types during bulk manufacturing steps, as well as segregation from other products manufactured in the same facility. Sufficient cleaning-validation and product-changeover data should be available to evaluate procedures. The antigen-manufacturing process should be validated to demonstrate production consistency. Typically, three consecutive lots per HPV type are required.

A.2.1 **Characterization of the antigen**

Characterization of HPV antigen is performed on lots produced during vaccine development, including the process-validation batches.
The protein composition should be established by techniques such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or mass spectrometry. The SDS-PAGE bands should be identified by sensitive staining techniques and where possible by specific antibodies, or mass spectrometry could be used to confirm the presence of the expected products of the L1 protein. The identity of the protein should be established by peptide mapping and/or analysis of the terminal amino acid sequences.

Since it is known that conformational epitopes are essential for efficacy, it is necessary that the morphological characteristics of the VLPs and degree of aggregation should be determined. VLP characterization may be done by additional appropriate methods.

A.3  Control of source materials
A.3.1  Cell cultures for antigen production
The use of any cell line should be based on a cell bank system (7, 37). Only cells that have been approved and registered with the NRA should be used to produce HPV L1 VLP vaccines. The NRA should be responsible for approving the cell bank or seed lot. An appropriate history of the cell bank should be provided. With regard to cell cultures, the maximum number of passages (or population doublings) allowable from the MCB, through the WCB, and through the production in cells should be approved by the NRA.

A.3.1.1  Recombinant yeast and bacteria cells
The characteristics of the parental cells and the recombinant production strain (parental cell transformed with the recombinant expression construct) should be fully described and information should be given on the testing for adventitious agents and on gene homogeneity for the MCB and WCB. A full description of the biological characteristics of the host cell and expression vectors should be given. This should include genetic markers of the host cell, the construction, genetics and structure of the expression vector, and the origin and identification of the gene that is being cloned. Some techniques (for example, deep sequencing) allow for the entire construct to be examined, while others (for example, restriction enzyme analysis) allow for assessment of segments (30, 38). The molecular and physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail (38).

The nucleotide sequence of the gene insert and the adjacent segments of the vector and restriction-enzyme mapping of the vector containing the gene insert should be provided as required by the NRA.

Cells must be maintained in a frozen state that allows for recovery of viable cells without alteration of genotype. The cells should be recovered from the frozen state, if necessary in selective media, such that the genotype and
phenotype consistent with the recombinant (modified) host and vector are maintained and clearly identifiable. Cell banks should be identified and fully characterized by means of appropriate tests.

Data – for example on plasmid restriction enzyme mapping, nutritional requirements or antibiotic resistance (if applicable) – that demonstrate the genetic stability of the expression system during passage of the recombinant WCB up to or beyond the passage level used for production should be provided to and approved by the NRA. Any instability of the expression system occurring in the seed culture or after a production-scale run should be documented. Stability studies should also be performed to confirm cell viability after retrieval from storage, maintenance of the expression system, etc. These studies may be performed as part of their routine use in production or may include samples taken specifically for this purpose.

A.3.1.1 Tests on recombinant yeast and bacteria MCB and WCB

MCBs and WCBs should be tested for the absence of bacterial and fungal contamination by appropriate tests, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (39), or by a method approved by the NRA, to demonstrate that only the bacteria or yeast production strain is present and that the MCB and WCB are not contaminated with other bacteria or fungi.

A.3.1.2 Insect cells

If insect cells are used, cell substrates and cell banks should conform with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7), as appropriate to insect cells, and should be approved by the NRA.

The MCB is made in sufficient quantities and stored in a secure environment, and is used as the source material for making the manufacturer's WCB. In normal practice an MCB is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the NRA, at which point the cells are combined to give a single pool which is distributed into ampoules and preserved cryogenically to form the WCB. WCBs of insect cells may be used for recombinant baculovirus seed lot production and antigen expression.

A.3.1.2.1 Tests on insect cell MCB and WCB

Tests on the MCB and WCB should be performed in accordance with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of
cell banks (7). It is important to show that the cell banks are free from bacteria, fungi, mycoplasmas, mycobacterium species (spp.), and adventitious agents relevant to the species that may be present in raw materials used in its derivation. For insect cell lines, special emphasis is put on potential insect-borne human pathogens (for example, arboviruses).

Insect viruses have not been well characterized compared with other potential adventitious agents, and there is therefore less information about them – and specifically about their infectivity, replicative life-cycles and pathogenicity, if any. It should be borne in mind that infection of insect cells with some insect viruses may occur without showing cytopathic effect. Tests may include specific nucleic acid amplification technique (NAT) tests such as polymerase chain reaction (PCR) and other nonspecific tests such as electron microscopy and co-cultivation. The specificity and sensitivity of assays should be determined by the manufacturer and approved by the NRA.

Full characterization may be performed on either the MCB or on the WCB, with more-limited testing on the other, depending on the strategy chosen for testing (7). Scientific advice on the testing strategy should be sought from the NRA.

A.3.1.3 Recombinant mammalian cells

If mammalian cells are used, the cell substrates and cell banks should conform with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7) and the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (38), and should be approved by the NRA.

A.3.2 Recombinant baculovirus master and working seeds for antigen production

The recombinant baculovirus expression vector contains the coding sequence of the HPV protein antigen. The recombinant baculovirus used in the production of HPV vaccines should be identified by historical records, which will include information on the origin and identity of the gene being cloned, and on the construction, genetics and structure of the baculovirus expression vector(s).

The production of vaccine should be based on the recombinant baculovirus master seed lot and working seed lot system. Recombinant baculovirus seed lots should be stored in a dedicated temperature-monitored refrigerator at a temperature that ensures stability and security.

Only recombinant baculovirus seed lots that are approved by the NRA should be used. The recombinant baculovirus master seed lot should be made
in sufficient quantities to last the lifetime of the vaccine product and should be stored in a secure environment, preferably in two geographically separate locations. The master seed lot is used as the source material for making the manufacturer’s recombinant baculovirus working seed lots. Either the virus master seed lots or the virus working seed lots should be fully characterized and tested extensively for adventitious agents, while the other may be subjected to more-limited testing. The testing strategy and seed lots should be approved by the NRA.

The manufacturer’s recombinant baculovirus working seed lot is used in the production of inoculum intermediates and single antigen harvests and is prepared from the master recombinant baculovirus seed lot. It is recommended that a large lot of recombinant baculovirus working seed should be set aside as the basic material that the manufacturer should use for the preparation of each batch of vaccine. The recombinant baculovirus working seed lot should be prepared by a defined number of passages from the recombinant baculovirus master seed lot using a method and a passage level from the original virus seed approved by the NRA. Once the acceptable passage level of the working seed lot is established, it may not be changed in making future lots of working seed without approval from the NRA.

A.3.2.1 Tests on recombinant baculovirus master and working seed lots

The expression construct should be analysed using NAT in conjunction with other tests performed on the purified recombinant protein for assuring the quality and consistency of the expressed HPV L1 antigens. The genetic stability and stability of expression of the expression construct should be demonstrated from the baculovirus master seed up to at least the highest passage level used in production, but preferably beyond this level (30, 38).

A.3.2.1.1 Identity

Each baculovirus master and working seed lot should be identified by the HPV type of the inserted gene using an appropriate method such as PCR. The tests should be approved by the NRA.

A.3.2.1.2 Sterility tests for bacteria, fungi and mycoplasmas

Each recombinant baculovirus seed lot should also be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests, as specified in Part A of the WHO General requirements for the sterility of biological substances, sections 5.2 (39) and 5.3 (40) or by a method approved by the NRA. In addition, the recombinant baculovirus seed lot should be tested for insect mollicutes (mycoplasma) such as spiroplasma, entomoplasma and mesoplasma.
NAT alone or in combination with cell culture, with an appropriate
detection method, may be used as an alternative to one or both of the
compendial mycoplasma-detection methods following suitable validation
and agreement from the NRA (7).

A.3.2.1.3 Tests for adventitious viruses

Each recombinant baculovirus seed lot should be tested in cell cultures for
adventitious viruses appropriate to the origin and the passage history of the seed
baculovirus. For tests on recombinant baculovirus-permissive indicator cells,
the neutralization of baculovirus is necessary. Antisera used for this purpose
should be free from antibodies that may neutralize adventitious viruses, and
should preferably be generated by the immunization of specific-pathogen-free
animals with an antigen made from a source (other than the production cell
line) which has itself been tested for freedom from adventitious agents. The
inoculated indicator cells should be examined microscopically for cytopathic
changes. At the end of the examination period, the cells should also be tested
for haemadsorbing viruses (see section A.4.2.1.1 below). It should be noted
that infection of indicator cells by insect viruses may not reveal any cytopathic
effect. Additional tests such as PCR, electron microscopy and co-cultivation
may therefore be performed. It is important to show that recombinant
baculovirus seeds are free of adventitious agents relevant to the species used
in their derivation with a special emphasis on potential insect-borne human
pathogens (for example, arboviruses). The specificity and sensitivity of assays
should be determined by the manufacturer and approved by the NRA.

In general, recombinant baculovirus seeds should be assessed for
absence of adventitious agents that may have been introduced during their
production, including those that may be present in the source materials used at
each of the production stages of the master and working virus seed lots. Each
virus master or working seed lot should also be tested in animals such as guinea-
pigs and/or mice. For details of these tests, see the WHO Recommendations
for the evaluation of animal cell cultures as substrates for the manufacture of
biological medicinal products and for the characterization of cell banks (7).

New molecular methods with broad detection capabilities are being
developed for the detection of adventitious agents. These methods
include: (a) degenerate NAT for whole virus families, with analysis of the
amplicons by hybridization, sequencing or mass spectrometry; (b) NAT
with random primers followed by analysis of the amplicons on large
oligonucleotide microarrays of conserved viral sequencing or digital
subtraction of expressed sequences; and (c) high-throughput or deep
sequencing. These methods may be used to supplement existing methods
or as alternative methods to in vivo and/or in vitro tests after appropriate
validation and approval by the NRA (7).
A.3.2.1.4 **Test for mycobacterium spp.**

Each recombinant baculovirus seed lot should be tested for mycobacterium spp. The test method and specifications should be approved by the NRA.

A.3.2.1.5 **Tests on control cells used for production of seeds**

Tests on control cell cultures should be undertaken as described below in section A.4.2.1.

A.3.2.1.6 **Recombinant baculovirus concentration**

Each recombinant baculovirus seed lot should be assayed for infectivity in a sensitive assay in an insect cell culture system. The detailed procedures for carrying out the tests and for interpreting the results should be those approved by the NRA.

A.3.3 **Cell culture medium**

If serum is used for the propagation of cells, it should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas – as specified in Part A of the WHO General requirements for the sterility of biological substances, sections 5.2 (39) and 5.3 (40) – as well as freedom from adventitious viruses.

Detailed guidance on detecting bovine viruses in serum for establishing MCBs and WCBs are given in Appendix 1 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7) and should be applied as appropriate. The guidance provided on detecting bovine viruses in serum for establishing the cell banks may also be applicable to production cell cultures. As an additional monitor of quality, sera may be examined for endotoxin. Gamma irradiation may be used to inactivate potential contaminant viruses, while recognizing that some viruses are relatively resistant to gamma irradiation.

Whatever the process used, the validation study has to determine the consistency and effectiveness of the process while maintaining serum performance. The use of non-inactivated serum should be justified and is not advised without strong justification. The non-inactivated serum must meet the same criteria as the inactivated serum when tested for sterility and absence of mycoplasmal and viral contaminants.

The source(s) of animal components used in culture medium should be approved by the NRA. These components should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (41).

Bovine or porcine trypsin used for preparing cell cultures should be tested and found free of bacteria, fungi, mycoplasmas and adventitious viruses,
as appropriate. The methods used to ensure this should be approved by the NRA. The source(s) of trypsin of bovine origin, if used, should be approved by the NRA and should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (41).

In some countries, irradiation is used to inactivate potential contaminant viruses. If irradiation is used it is important to ensure that a reproducible dose is delivered to all batches and to the component units of each batch. The irradiation dose must be low enough to allow the biological properties of the reagents to be retained but high enough to reduce virological risk. Therefore, irradiation cannot be considered a sterilizing process (7). The irradiation method should be validated and approved by the NRA.

Recombinant trypsin is available and should be considered; however, it should not be assumed to be free from risk of contamination and should be subject to the usual considerations for any reagent of biological origin (7).

Human serum should not be used. If human serum albumin is used at any stage of product manufacture, the NRA should be consulted regarding the requirements, as these may differ from country to country. As a minimum, the use of human serum albumin should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (42). In addition, human albumin and materials of animal origin should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (41).

Penicillin and other beta-lactams should not be used at any stage of the manufacture because they are highly sensitizing substances.

Other antibiotics may be used in the manufacture provided that the quantity present in the final lot is acceptable to the NRA.

Non-toxic pH indicators may be added (for example, phenol red at a concentration of 0.002%). Only substances that have been approved by the NRA may be added.

A.4 Control of HPV VLP production
A.4.1 Control of HPV VLP production up to single antigen harvest in yeast and bacterial expression systems
A.4.1.1 Microbial purity

Microbial purity in each fermentation vessel should be monitored at the end of the production run by methods approved by the NRA.
Any agent added to the fermentor or bioreactor with the intention to feed cells or to induce or increase cell density should be approved by the NRA. No antibiotics should be added at any stage of manufacturing unless approved by the NRA.

A.4.2 Control of HPV VLP production up to single antigen harvest in recombinant baculovirus systems in insect cells

Cell cultures are expanded to an appropriate scale and are then inoculated with recombinant baculovirus at a defined multiplicity of infection (MOI). After adsorption, the cell cultures are fed with maintenance medium and incubated within a defined temperature range and for a defined period of time.

The range of MOI, temperature, pH and incubation period will depend on the insect cell substrate and the specific characteristics of the recombinant baculovirus strain. A defined range for the MOI should be established by the manufacturer and approved by the NRA.

A single antigen harvest is obtained within a defined time period post-inoculation. Several single antigen harvests may be pooled. If multiple single antigen harvests are pooled, each single antigen harvest should be sampled for testing, stabilized and stored under suitable conditions until pooling. No antibiotics should be added at the time of harvesting or at any later stage of manufacture. Samples of single harvest pools should be taken for testing and should be stored at a temperature of −60 °C or below.

A.4.2.1 Tests on control cell cultures

When the insect cell suspension is grown to the scale needed for production, but prior to infection with the recombinant baculovirus, an amount of processed cell suspension equivalent to at least 5% or 500 ml of cell suspension (whichever is greater) should be used to prepare control cultures of uninfected cells. If bioreactor technology is used, the size and treatment of the cell sample to be examined should be approved by the NRA.

The control cell cultures should be examined microscopically for the morphological changes of the cells attributable to the presence of adventitious agents for at least 14 days after the day of inoculation of the production cultures, or at the time of final virus harvest if this is later. The control cell cultures should be incubated under conditions that are essentially similar to those used for the production cultures, with the agreement of the NRA. For insect cells, the above incubation time may not apply because of the specificities of cells cultivated in suspension but it should be not less than the time of collection of the single antigen harvest. At the end of the examination period, fluids collected from the control cell culture from each single antigen harvest should be tested for the presence of adventitious agents as described below. Samples that are not tested immediately should be stored at −60 °C or below.
If any test shows evidence of the presence of adventitious agents in control cell cultures, the single antigen harvests prepared from these cultures should not be used for HPV VLP production.

For the test to be valid at least 80% of the control cells should still survive by the end of the test period and any losses should be due to nonspecific or accidental reasons.

A.4.2.1.1 Tests for haemadsorbing viruses

At the end of the observation period at least 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the red blood cells have been stored the duration of storage should not have exceeded 7 days, and the temperature of storage should have been in the range of 2–8 °C.

In some countries, the NRA requires that additional tests for haemadsorbing viruses are to be performed using red blood cells of other species, including from humans (blood group O), monkeys and/or chickens (or other avian species).

All haemadsorption tests should be read after incubation for 30 minutes at 0–4 °C, and again after incubation for a further 30 minutes at 20–25 °C.

If a test with monkey red blood cells is performed, readings should also be taken after a final incubation for 30 minutes at 34–37 °C.

For cells cultivated in suspension, the test for presence of haemadsorbing viruses is not technically feasible. A test for presence of haemagglutinating agents using guinea-pig red blood cells is therefore required with spent control cell culture fluid.

For the tests to be valid not more than 20% of the culture vessels should have been discarded by the end of the test period and any losses should be due to nonspecific or accidental reasons.

A.4.2.1.2 Tests for other adventitious agents

At the end of the observation period a sample of the pooled fluid and/or cell lysate from each group of control cell cultures should be tested for adventitious agents. For this purpose, 10 ml of each pool should be tested in the same cell line as used for the production of virus, but not the same batch of cells as those used for the production of vaccine. In addition, 10 ml samples of each pool should also be tested in human cells and in a simian kidney cell line.

Each sample should be inoculated into culture vessels of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cells should be at least 3 cm².
per ml of pooled fluid. At least one culture vessel of each kind of cell culture should remain un-inoculated as a control.

The inoculated cultures should be incubated at the appropriate growth temperature and should be observed for cytopathic effects for a period of at least 14 days.

For the tests to be valid not more than 20% of the culture cells should have been discarded by the end of the test period and any losses should be due to nonspecific or accidental reasons.

Some NRAs require that these cells should be tested for the presence of haemadsorbing viruses at the end of the observation period.

**A.4.2.1.3 Test for identity of insect cells**

At the production level, the cells should be identified by means of tests approved by the NRA. Suitable methods include, but are not limited to, biochemical tests (for example, isoenzyme analyses), cytogenetic tests (for example, for chromosomal markers) and tests for genetic markers (for example, DNA fingerprinting or PCR).

**A.4.3 Control of HPV VLP production up to single antigen harvest in mammalian cells**

If applicable, tests on control cells for identity and adventitious agents should be performed in accordance with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7).

**A.4.4 Control of single antigen harvests**

**A.4.4.1 Storage and intermediate hold times**

Prior to and during the purification process, the cell suspension or supernatant fluid should be maintained under conditions shown by the manufacturer to allow them to retain the desired biological activity. Hold times should be approved by the NRA.

**A.4.4.2 Tests on single antigen harvest or single harvest pool**

If appropriate, tests may be conducted on a single antigen harvest or on a pool of single antigen harvests. The protocol should be approved by the NRA.

**A.4.4.2.1 Sampling**

Samples required for the testing of single antigen harvests or single harvest pools should be taken immediately on harvesting and before further processing. If tests for sterility and adventitious agents, as described below in sections
A.4.4.2.2 and A.4.4.2.4, are not performed immediately then the samples taken for these tests should be kept at a temperature of \(-60\, ^\circ\text{C}\) or below and subjected to no more than one freeze–thaw cycle.

### A.4.4.2.2 Sterility tests for bacteria, fungi and mycoplasmas

Each single antigen harvest or single harvest pool should be shown to be free from bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (39).

Harvests from bacterial expression systems could be positive for bacterial contamination. Therefore, an alternative method such as the microbial limits test might be appropriate for addressing culture purity. Such testing should be approved by the NRA.

In addition to sterility tests for bacteria and fungi, each single antigen harvest or single harvest pool should also be shown to be free from mycoplasmal contamination by appropriate tests as specified in Part A, section 5.3 of the WHO General requirements for the sterility of biological substances (40) if insect or mammalian cells are used in production, or by a method approved by the NRA.

NAT alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma-detection methods following suitable validation and agreement from the NRA (7).

### A.4.4.2.3 Test for identity of HPV types

Each single antigen harvest or single harvest pool should be identified as the appropriate HPV type by immunological assay or by a molecular biology based assay (for example, hybridization or PCR). The tests should be approved by the NRA. Alternatively, the identity can be confirmed as part of testing of the purified antigen.

### A.4.4.2.4 Tests for adventitious agents if insect or mammalian cells are used in production

Each single antigen harvest or single harvest pool should be tested for adventitious viruses in cell cultures selected for their appropriateness to the origin and passage history of the insect cell substrate and recombinant baculovirus or the mammalian cell substrate. These cell cultures should include, as a minimum, a monkey kidney cell line and a human cell line. Antisera used for the purpose of neutralizing the recombinant baculovirus should be free from antibodies that may neutralize adventitious viruses, and should preferably be generated by the immunization of specific-pathogen-free animals with an antigen made...
from a source (other than the production cell line) which has itself been tested for freedom from adventitious agents. The inoculated indicator cells should be examined microscopically for cytopathic changes. At the end of the examination period, the cells should also be tested for haemadsorbing viruses (see section A.4.2.1.1 above).

Additional testing for specific adventitious viruses may be performed, for example by using PCR amplification techniques.

A.5  Control of purified monovalent antigen bulk
The purification process can be applied to a single antigen harvest, part of a single antigen harvest or a pool of single antigen harvests, and should be approved by the NRA. The maximum number of harvests that may be pooled should also be approved by the NRA. Adequate purification may require several purification steps based on different biophysical and/or biochemical principles and may involve disassembly and reassembly of VLPs. The entire process (sequence of process steps) used for the purification of the VLPs should be appropriately validated, as described above in section A.2, and should be approved by the NRA. Any reagents added during the purification processes (such as DNase) should be documented.

The purified monovalent antigen bulk can be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity. Intermediate hold times should be approved by the NRA.

A.5.1  Tests on the purified monovalent antigen bulk
Purified monovalent antigen bulks should be subjected to the tests listed below. Some tests may be omitted if performed on the adsorbed monovalent antigen bulk. All quality control release tests and specifications for purified monovalent antigen bulk, unless otherwise justified, should be validated by the manufacturer and approved by the NRA.

A.5.1.1  Identity
Each purified monovalent antigen bulk should be identified as the appropriate HPV antigen type by a method suitable for distinguishing between HPV types (for example, an immunological assay). The test for antigen content may also serve as the identity test.

A.5.1.2  Purity
The degree of purity of each purified monovalent antigen bulk, and levels of residual host cell protein, should be assessed by suitable methods. One suitable method for analysing the proportion of potential contaminating proteins in
the total protein of the preparation is separation of the proteins by SDS-PAGE under reducing denaturing conditions. The protein bands within the gel should be identified by sensitive staining techniques. The protein in each band should be quantified by densitometric analysis at an appropriate level of sensitivity, in order to measure the degree of purity.

Host cell protein detection by expression-specific and sensitive EIA methods may be developed.

A.5.1.3 Protein content
Each purified monovalent antigen bulk should be tested for the total protein content using a suitable method.

Alternatively, the total protein content may be calculated from measurement of an earlier process intermediate.

A.5.1.4 Antigen content
The antigen content should be measured on the purified monovalent antigen bulk or the adsorbed monovalent antigen bulk (see section A.6.3.7 below) by an appropriate method which is type specific.

The ratio of antigen content to protein content may be calculated and monitored for each purified monovalent antigen bulk.

International Standards and Reference Reagents for the control of HPV VLP vaccine antigen content are not available. Therefore, product-specific reference preparations may be used.

A.5.1.5 Sterility tests for bacteria and fungi
Each purified monovalent antigen bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (39), or by a method approved by the NRA. Alternatively, this test can be performed on the related adsorbed monovalent antigen bulks if the purified bulk is not stored prior to adsorption.

A.5.1.6 Percentage of intact L1 monomer
The integrity of L1 protein is a critical quality parameter and should be carefully monitored. The percentage of intact L1 protein of each purified monovalent antigen bulk should be assessed by suitable methods. The purity assay (see section A.5.1.2 above) may also serve to assess the integrity of the L1 monomer.

The percentage of intact L1 monomer is the ratio of the intact L1 monomer to the total protein expressed as a percentage – that is, intact L1 monomer/(total L1 + total non-L1) x 100.
A.5.1.7 VLP size and structure
The size and structure of the VLPs are to be established and monitored. This test may be omitted for routine lot release once consistency of production has been established, with the agreement of the NRA.

Suitable methods for assessing VLP size and structure include dynamic light scattering, size-exclusion chromatography – high-performance liquid chromatography (SEC–HPLC) and transmission electron microscopy (TEM).

A.5.1.8 Tests for reagents used during purification or other phases of manufacture
A test should be carried out to detect the presence of any potentially hazardous reagents used during manufacture, using a method(s) approved by the NRA. This test may be omitted for routine lot release upon demonstration that the process consistently eliminates the reagent from the purified monovalent antigen bulks.

A.5.1.9 Tests for residual DNA derived from the expression system
The amount of residual host cell DNA derived from the expression system should be determined in each purified monovalent antigen bulk by suitably sensitive methods. The level of host cell DNA should not exceed the maximum level agreed with the NRA, taking into consideration issues such as those discussed in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7).

These tests may be omitted for routine lot release upon demonstration that the process consistently inactivates the biological activity of the residual DNA or reduces the amount and size of the contaminating residual DNA present in the purified monovalent antigen bulks, subject to the agreement of the NRA.

A.5.1.10 Bovine serum albumin content
If bovine serum is used in mammalian or insect cell cultures for production then residual bovine serum albumin content should be measured and a maximum permitted concentration should be set, and approved by the NRA.

A.5.1.11 Test for viral clearance
When an insect or mammalian cell substrate is used for the production of HPV antigens, the production process should be validated in terms of its capacity to remove and/or inactivate adventitious viruses – as described in the Q5A guidelines (43) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. This testing
is performed during vaccine manufacturing development or as part of process validation and is not intended as an assessment for lot release.

If a replicating viral vector such as a baculovirus is used then the production process should be validated for its capacity to eliminate (by removal and/or inactivation) residual recombinant virus.

A.6 Control of adsorbed monovalent antigen bulk

A.6.1 Addition of adjuvant

The purified monovalent antigens may be adsorbed onto an adjuvant such as an aluminium salt, in which case the adjuvant and the concentration used should be approved by the NRA. If an alternative or additional adjuvant such as MPL is used, this should also be approved by the NRA.

If a novel adjuvant is used that does not involve adsorption of the VLP to the adjuvant, the term “adjuvanted monovalent antigen bulk” may be used.

A.6.2 Storage

Until the adsorbed monovalent antigen bulk is formulated into the final bulk, the suspension should be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity. Hold times should be approved by the NRA.

A.6.3 Tests on adsorbed monovalent antigen bulk

All tests and specifications for adsorbed monovalent antigen bulk, unless otherwise justified, should be approved by the NRA.

A.6.3.1 Sterility tests for bacteria and fungi

Each adsorbed monovalent antigen bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (39), or by an alternative method approved by the NRA.

A.6.3.2 Bacterial endotoxins

Each adsorbed monovalent antigen bulk should be tested for bacterial endotoxins using a method approved by the NRA.

If it is inappropriate to test the adsorbed monovalent antigen bulk, the test should be performed on the purified antigen bulk prior to adsorption, subject to the approval of the NRA.
A.6.3.3 Identity
Each adsorbed monovalent antigen bulk should be identified as the appropriate HPV antigen type by a method suitable for distinguishing between HPV types (for example, an immunological assay). The test for antigen content may also serve as the identity test.

A.6.3.4 Adjuvant concentration
Adsorbed monovalent antigen bulk may be assayed for adjuvant content until production consistency is demonstrated.

A.6.3.5 Degree of adsorption
The degree of adsorption (completeness of adsorption) of each adsorbed monovalent antigen bulk should be assessed, if applicable. This test may be omitted upon demonstration of process consistency, subject to the agreement of the NRA.

A.6.3.6 pH
The pH value of the adsorbed monovalent antigen bulk may be monitored until production consistency is demonstrated, subject to the agreement of the NRA.

A.6.3.7 Antigen content
The antigen content of the adsorbed monovalent antigen bulk should be measured using appropriate methods. If this test is conducted on purified monovalent antigen bulk it may be omitted from the testing of the adsorbed monovalent antigen bulk.

International Standards and Reference Reagents for the control of HPV VLP vaccine antigen content are not available. Therefore, product-specific reference preparations may be used.

A.7 Control of final bulk
The final bulk should be aseptically prepared by combination of the adsorbed monovalent antigen bulks which pass the tests specified in section A.6.3 above. The antigen concentration in the final formulation should be sufficient to ensure that the dose is consistent with that shown to be safe and effective in human clinical trials. Should an adjuvant be added to the vaccine formulation, this adjuvant and the concentration used should be approved by the NRA.

The operations necessary for preparing the final bulk should be conducted in such a manner as to avoid contamination of the product. In preparing the final bulk vaccine, any substances such as diluents, stabilizers
or adjuvants that are added to the product should have been shown to the satisfaction of the NRA not to impair the safety and efficacy of the vaccine at the concentration used. The final bulk should be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity until it is filled into containers.

A.7.1 Tests on the final bulk
All tests and specifications for final bulk, unless otherwise justified, should be approved by the NRA.

A.7.1.1 Sterility tests for bacteria and fungi
Each final bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (39), or by a method approved by the NRA.

A.7.1.2 Adjuvant content
Each final bulk should be assayed for adjuvant content.

Where aluminium compounds are used, the amount of aluminium should not exceed 1.25 mg per single human dose.

An example of a suitable test for an adjuvant such as MPL is gas chromatography.

Tests for adjuvant content on the final bulk may be omitted if conducted on each final lot derived from the final bulk.

A.7.1.3 Degree of adsorption
The degree of adsorption to the adjuvant (completeness of adsorption) of each antigen present in each final bulk should be assessed, if applicable (for example, if the adjuvant is aluminium salts).

This test may be omitted upon demonstration of process consistency or if performed on the final lot.

A.7.1.4 Preservative content
The final bulk may be tested for the presence of preservative, if added. The method used and the permitted concentration should be approved by the NRA.

A.7.1.5 Potency
The potency of each final bulk should be assessed with an appropriate in vivo or in vitro method. If an in vivo potency test is used to test final lots, this test may be omitted on the final bulk. The methods for detection of antibodies to HPV VLPs
and the analysis of data should be approved by the NRA. The vaccine potency should be compared with that of a reference preparation; the NRA should determine the limits of potency and approve the reference preparation used.

For ethical reasons, it is desirable to apply the 3R principles (Replacement, Reduction, Refinement) to the use of animals, where scientifically appropriate (44).

A.7.1.6 Osmolality
The osmolality of the final bulk may be tested. The osmolality test may be omitted if performed on the final lot.

Alternative tests (for example, freezing point) may be used as surrogate measures for ionic strength/osmolality.

A.8 Filling and containers
The requirements concerning filling and containers given in WHO good manufacturing practices for biological products (36) should apply to vaccine filled in the final form.

Care should be taken to ensure that the materials of which the container – and if applicable the transference devices and closure – are made do not adversely affect the quality of the vaccine.

Manufacturers should provide the NRA with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

A.9 Control tests on the final lot
The following tests should be performed on each final lot (that is, in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers from each final lot by means of validated methods approved by the NRA. All tests and specifications, including methods used and permitted concentrations, unless otherwise justified, should be approved by the NRA.

A.9.1 Inspection of containers
Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities should be discarded and recorded for each relevant abnormality. A limit should be established for the percentage of containers rejected.

A.9.2 Appearance
The appearance of the vaccine should be described with respect to its form and colour.
A.9.3 **Identity**
All antigens present in the final lot should be identified by appropriate methods. The potency test may serve as the identity test.

A.9.4 **Sterility tests for bacteria and fungi**
Each final lot should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (39), or by a method approved by the NRA.

A.9.5 **pH and osmolality**
The pH value and osmolality of the final lot should be tested. The osmolality test may be omitted if performed on the final bulk. The osmolality test may also be omitted for routine lot release upon demonstration of product consistency, subject to the approval of the NRA.

An alternative test (for example, freezing point) may be used as a surrogate measure for ionic strength/osmolality.

A.9.6 **Preservatives**
Each final lot should be tested for the presence of preservative, if added.

A.9.7 **Test for pyrogenic substances**
Each final lot should be tested for pyrogenic substances. Where appropriate, tests for endotoxin (for example, the limulus amebocyte lysate (LAL) test) should be performed. However, where there is interference in the test – for example, because of the addition of an immunostimulant such as MPL – a test for pyrogens in rabbits should be performed.

A suitably validated monocyte-activation test may also be considered as an alternative to the rabbit pyrogen test.

The test is conducted until consistency of production is demonstrated, subject to the agreement of the NRA.

A.9.8 **Adjuvant content**
Each final lot should be assayed for adjuvant content, if applicable. Where aluminium compounds are used, the amount of aluminium should not exceed 1.25 mg per single human dose.
A.9.9 **Protein content**
The protein content should be determined. Alternatively this may be calculated from an earlier process intermediate.

A.9.10 **Degree of adsorption**
The degree of adsorption to the adjuvant (completeness of adsorption) of each antigen present in each final vaccine lot should be assessed, if applicable, and the limit should be approved by the NRA.

This test may be omitted for routine lot release upon demonstration of product consistency, subject to the approval of the NRA.

A.9.11 **Potency**
An appropriate quantitative test for potency by an in vivo or in vitro method should be performed on samples that are representative of each final vaccine lot. The method and the analysis of data from potency tests should be approved by the NRA. The vaccine potency should be compared with that of a reference preparation, and the limits of potency should be agreed with the NRA. The NRA should approve the reference preparation used. If an in vivo potency test is used, this test may be omitted on the final bulk. The method of testing for antigen potency in an in vitro test could be quantitative with respect to the antigen content or relative to a reference preparation.

Because of the diversity in the reactivity of vaccines containing HPV VLPs produced by different manufacturing techniques and differences in the adjuvants used for the vaccine formulation, it is unlikely that International Standards will be suitable for the standardization of assays of vaccines from all manufacturers. Consequently, International Standards will not be developed for the potency of each HPV type. Manufacturers should establish a product-specific reference preparation that is traceable to a lot of vaccine, or bulks used in the production of such a lot, which has been shown to be efficacious in clinical trials. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters and the reference vaccine should be replaced when necessary. An acceptable procedure for replacing reference vaccines should be in place (45, 46).

A.9.12 **General safety (innocuity) test**
The need to test the final lots of the HPV vaccine for unexpected toxicity (also known as abnormal toxicity) should be agreed with the NRA.

Some countries no longer require this test (47).
A.10  **Records**
The requirements given in WHO good manufacturing practices for biological products (36) should apply.

A.11  **Retained samples**
The requirements given in WHO good manufacturing practices for biological products (36) should apply.

A.12  **Labelling**
The requirements given in WHO good manufacturing practices for biological products (36) should apply, with the addition of the following information.
The label on the carton, the container or the leaflet accompanying the container should state:

- that the vaccine has been prepared from recombinant yeast, bacterial cells, mammalian cells or recombinant baculovirus/insect cells;
- the type of HPV (from which L1 VLPs were derived) present in the preparation;
- the potency per dose;
- the number of doses, if the product is issued in a multiple-dose container;
- the name and maximum quantity of any antibiotic present in the vaccine;
- the name and concentration of any preservative added;
- the name and concentration of any adjuvant added;
- the temperature recommended during storage and transport;
- the expiry date;
- any special dosing schedules.

A.13  **Distribution and transport**
The requirements given in WHO good manufacturing practices for biological products (36) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (48).
A.14 Stability testing, storage and expiry date

A.14.1 Stability testing

Adequate stability studies form an essential part of vaccine development. Current guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (49). Stability testing should be performed at different stages of production, namely on single antigen harvests or single harvest pools, purified monovalent antigen bulk, adsorbed monovalent antigen bulk, final bulk (whenever materials are stored before further processing) and final lot. Stability-indicating parameters appropriate to the stage of production should be defined or selected. A shelf-life should be assigned to all in-process materials during vaccine production – particularly intermediates such as single antigen harvests, purified monovalent antigen bulk and final bulk.

The stability and expiry date of the vaccine in its final container, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA using final containers from at least three final lots made from different adsorbed monovalent antigen bulks.

Given the complexity of these multivalent vaccines, other approaches may be used with the approval of the NRA.

Accelerated stability tests may be undertaken to give additional information on the overall characteristics of a vaccine, and may also aid in assessing comparability when the manufacturer plans to change aspects of manufacturing.

The formulation of vaccine antigens and adjuvant (if used) must be stable throughout the shelf-life of the vaccine. Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (49). Data should be provided to the NRA in accordance with local regulatory requirements.

The final stability-testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection and sharing of stability data, and criteria for rejecting vaccine(s).

A.14.2 Storage conditions

The final lot should be kept at 2–8 °C. If other storage conditions are used, they should be fully validated and approved by the NRA. The vaccine should have been shown to maintain its potency for a period equal to that between the date of release and the expiry date. During storage, liquid vaccines should not be frozen.
If a vaccine has been shown to be stable at temperature ranges higher than the approved 2–8 °C range, it may be stored in a controlled temperature chain for a defined period of time, subject to approval by the NRA.

A.14.3 Expiry date

The expiry date should be based on the shelf-life supported by stability studies and should be approved by the NRA. The expiry date should be based on the date of blending of final bulk, date of filling or the date of the first valid potency test on the final lot.

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals are inoculated.

Part B. Nonclinical evaluation of recombinant HPV VLP vaccines

Details of the design, conduct, analysis and evaluation of nonclinical studies are available in the WHO guidelines on nonclinical evaluation of vaccines (5). Further guidance on the general principles for nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines can be found in a separate WHO Guidelines document (8). In particular, this latter document should be consulted to determine the need for safety, pharmacology and bio-distribution studies, as well as the extent of characterizing of the adjuvant alone, should a novel adjuvant be introduced into the HPV vaccine formulation.

The guidance given in this current section will also apply to new L1 VLP vaccines containing other HPV types in addition to the types – 16/18 (± 6/11) – contained in the related licensed vaccine, and to new L1 VLP vaccines containing types 16/18 (± 6/11) with and without additional types.

Prior to the clinical testing of any new HPV vaccine in humans there should be extensive product characterization, proof-of-concept immunogenicity studies and safety testing in animals. The extent of nonclinical evaluation will depend on the complexity of the vaccine formulation, on a case-by-case basis. The following specific issues should be considered in the context of the development of an HPV L1 VLP-based vaccine.

B.1 Product characterization and process development

It is crucially important that vaccine production processes are appropriately standardized and controlled to ensure consistency in manufacturing, and in the collection of nonclinical data that may indicate potency and safety in humans. The extent of product characterization may vary according to the stage of development. The vaccine lots used in nonclinical studies should be adequately
representative of the formulation intended for use in clinical investigation and, ideally, should be the same lots as those used in clinical trials. If this is not feasible, the lots used in nonclinical studies should be comparable to clinical lots with respect to physicochemical characteristics and data, stability and formulation.

B.2 Pharmacodynamic studies

There is no adequate, relevant animal model for HPV infection as papillomaviruses are species specific. The proof-of-concept for the use of L1 VLP-based vaccines has been demonstrated in animal protection models using “homologous” viruses such as cottontail rabbit papillomavirus. These preclinical challenge studies in various animal models have indeed demonstrated that L1 VLPs are potent immunogens that induce high titres of neutralizing antibodies and protect against associated lesions (20, 21). Furthermore, transfer of serum from animals vaccinated with L1 VLPs provided protection in non-vaccinated animals challenged with the virus (20–22). One mechanism-of-protection study in a murine cervicovaginal model of infection using a surrogate virus or pseudovirion has indicated that exudation of systemic neutralizing antibodies into the wound bed and binding to the virus at the basement membrane probably account for the protection (22).

On the basis of these data:

- No further challenge studies need to be performed for monovalent or multivalent HPV L1 VLP vaccine.
- Neutralizing antibodies are probably the primary mediator of protection. It seems unlikely that CMI is substantially involved as a direct effector mechanism of protection; thus, this aspect does not need further elucidation to support regulatory approval.

It is recommended that the pharmacodynamic properties of an L1 VLP-based vaccine should be assessed through immunogenicity studies (for example, in rodents, rabbits and/or possibly in non-human primates) which should take into account:

- the evaluation and characterization of the serum neutralizing antibodies induced against each of the HPV L1 VLP types included in the proposed vaccine – if the HPV vaccine contains new types in addition to those in a licensed vaccine or a new vaccine formulation intended for an alternative route of administration (for example, aerosol), the inclusion of an additional arm(s) in the study design may generate supportive data;
- in the case of the inclusion of a specific adjuvant in the vaccine, the added benefit with respect to enhancing desired immune response (humoral and/or cellular – for example, involvement of T-helper
cells or induction of specific memory cells) to be studied in one or more relevant species (8);
- the generation of supportive data with respect to the relative ratio of the antigen and the adjuvant included in the vaccine formulation;
- the evaluation of serum cross-neutralizing antibodies to a broad range of HPV types not included but related to those in the proposed vaccine is optional (see section C.6 below).

B.3 Toxicology studies

Toxicology studies should be undertaken with the final vaccine formulation in accordance with WHO guidance (5). Such studies should be performed in relevant animal models, and should reflect the intended clinical use of the vaccine and may include the administration of doses prior to and during pregnancy (5). Because the target population for the HPV vaccines includes women of childbearing age, reproductive and developmental toxicity studies are required. Some NRAs will require submission of the pivotal data at the time of the marketing authorization application (8). Repeated-dose toxicity studies and developmental toxicity studies should include evaluation of the immune response in a group of vaccinated animals, in addition to appropriate toxicological end-points, taking into account existing WHO guidelines (5, 8). Other toxicity studies, such as an inhalation toxicity study, may be required if the vaccine is developed for administration through the aerosol route.

If a novel cell substrate (that is, a substrate that has not previously been used to produce a licensed human vaccine or biological product) is used for the production of an HPV VLP vaccine then safety aspects – such as potential immune responses elicited by residual host cell proteins – should be investigated in a suitable animal model. Such studies should be undertaken particularly if the final product contains an adjuvant that might enhance responses to low levels of residual proteins.

Part C. Clinical evaluation of recombinant HPV VLP vaccines

C.1 Introduction

Clinical studies should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (50) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6).

This section refers throughout to L1 VLP vaccines that contain at least HPV types 16 and 18 and considers the clinical data that may be generated both prior to initial licensure and post-licensure in different settings.
Three types of clinical data can provide an indication of the ability of a candidate HPV vaccine to prevent anogenital carcinomas:

- immunological data as discussed in section C.2 below;
- virological data as discussed in section C.3 below;
- histological data as discussed in section C.4 below.

Section C.5 summarizes the type(s) of data that may be used to demonstrate vaccine efficacy in different settings.

These four sections (C.2–C.5) take into account the fact that histological primary efficacy end-points have diminishing feasibility as a result of the impact of HPV vaccines on the occurrence of infections due to types 16 and 18 and the lower prevalence of other oncogenic HPV types in high-grade intraepithelial neoplasms and cancers. Experience gained with the first two HPV vaccines has supported the use of type-specific viral persistence as an alternative primary efficacy end-point where there has been no routine HPV vaccination or where uptake has been low. Neither histological nor viral-persistence end-points are likely to be feasible for types 16 and 18 in countries that have included HPV vaccines in their routine programmes with high uptake rates. These end-points are also not expected to be feasible for documenting efficacy against other HPV types that are much less commonly, and sometimes rarely, associated with premalignant lesions. Consequently, in some settings an assessment of vaccine efficacy can be based only on immunological data.

In addition:

- Section C.6 considers the data that could support claims for protection against HPV types that are not included in a candidate vaccine (that is, cross-protection).
- Section C.7 considers the assessment of safety within clinical studies.
- Section C.8 considers the assessment of antibody persistence, vaccine effectiveness and safety in routine use.

C.2 Immunological data

Naturally acquired neutralizing (that is, functional) antibody to HPV has been demonstrated to afford some type-specific protection (51). HPV L1 VLP vaccines are thought to protect against persistent viral infection by eliciting neutralizing antibody that can be measured in serum. Studies with intramuscular HPV L1 VLP vaccines have found an overall correlation between antibody levels in serum and in cervical mucosal fluids (52–56). However, the antibody concentrations in serum or at the site of infection that are required for protection are unknown – that is, no immune correlate of protection (ICP) has been established for HPV vaccines.
The initial assessment of immune responses to HPV L1 VLP vaccines should be based on measurement of neutralizing antibodies in serum using a pseudovirion-based neutralization assay. Measurement of antibody concentrations at anogenital sites (for example, in cervical mucosal fluid) is not required.

C.2.1 Assays

Throughout any one clinical development programme it is preferable that the same assays for determination of anti-HPV antibody are used and that testing is conducted at a designated central laboratory. Ideally, the same approach should apply to post-licensing investigations of antibody persistence. This uniformity becomes essential within any one study and when attempting to compare immune responses between studies. The assay (or assays, if a change in assay during clinical development was unavoidable) used to generate the immune-response data included in the application dossier should be fully validated. The details and results of the validation exercise(s) should be provided.

In vitro neutralizing antibody assays involve measurement of the inhibition of HPV pseudovirus infection of cultured cells and usually employ type-specific pseudovirions carrying a marker plasmid to allow infected cells to be scored easily. These neutralizing assays require expression plasmid constructs for L1 and L2 for each viral type, and assay standardization relies on use of the same source for these constructs. The WHO Human papillomavirus laboratory manual (24) includes a method for HPV neutralizing assays that has shown good inter-laboratory performance.

However, neutralization assays are labour intensive, technically complex and not currently amenable to high throughput. Therefore, following characterization of the neutralizing antibody response to a candidate HPV vaccine, the use of alternative assay methods that are less technically demanding (for example, type-specific competitive Luminex immunoassay (cLIA) or EIA) may be acceptable subject to demonstration of strong correlation between the results of these assays and neutralizing assays (57).

Competitive immunoassays utilizing neutralizing monoclonal antibodies that bind to conformational epitopes on L1 are type specific and sensitive, detect all immunoglobulin classes and do not measure antibodies to denatured L1 protein (57). However, only a subset of the total anti-VLP antibodies is measured since binding to only one neutralizing epitope is monitored. Therefore, if these assays are used it should be kept in mind that the results may under-represent the total level of protective antibodies in sera.

VLP-based EIAs require the use of conformationally intact VLPs as antigens to ensure type specificity. These assays detect all antibodies of a specific immunoglobulin class (generally immunoglobulin G) that bind to the VLPs. While both neutralizing and non-neutralizing antibodies are detected,
the correlation with neutralizing assays has been generally good because the strongest host response to vaccines developed to date is to neutralizing epitopes. Laboratories performing HPV serology testing have to prepare and conduct quality-control approaches for their own VLPs because no commercial assays are available. The inability of laboratories to access common key source reagents for serology assays presents significant challenges to the standardizing of HPV serology results.

International Standards for serum antibodies to HPV type 16 and HPV type 18 are available to help improve the comparability of results. The use of the parallel-line method with standards calibrated to the International Standard is described in the WHO Human papillomavirus laboratory manual (24) and has been shown to improve inter-laboratory comparisons. Antibody levels should be reported in International Units (IU) for HPV types for which an International Standard is available. It should be kept in mind that the comparison of titres between HPV types is not appropriate. For each assay the lower limit of detection (LLOD) and lower limit of quantification (LLOQ) should be clearly established, along with a justification of the cut-off applied to differentiate samples that are reported to be seropositive and seronegative.

C.2.2 Characterization of the immune response

During the initial phase of the clinical development programme it is recommended that the following should be documented for each HPV type in a candidate vaccine:

- immune responses to the vaccine, with a focus on seroconversion rates in subjects who were seronegative prior to vaccination;
- pre- to post-vaccination changes in geometric mean titres (GMTs) and reverse cumulative distributions (these should be presented separately for subjects who were seropositive or seronegative prior to vaccination).

The following should be evaluated in clinical studies, unless the sponsor demonstrates that the candidate vaccine is very similar to a licensed vaccine so that the same sera collection times and dose regimens may apply:

- The kinetics of the immune response (that is, changes in antibody levels in response to sequential doses) should be determined to support the timing of the post-vaccination serological primary end point.
- There should be an adequate exploration of immune responses to support the number of doses and dose interval selected for various age groups.
Inclusion of an adjuvant, whether novel or already included in licensed vaccine(s), should be supported by data demonstrating enhancement of the immune response to one or more HPV types, and by an assessment of the potential clinical significance of the antibody levels achieved in the target age range (8). Since there is no established ICP for HPV vaccines, the potential clinical significance of the effect of an adjuvant could be assessed by comparing antibody levels achieved by the candidate vaccine with and without the adjuvant with those elicited by a licensed vaccine for which efficacy has been documented (see section C.2.3 below).

If the adjuvant in a candidate vaccine is not identical to one in any type of licensed vaccine for which there is already substantial information available on the mechanism of immune enhancement, its effect on the humoral and cellular immune response (for example, involvement of T-helper cells or induction of specific memory cells) should be extensively characterized.

Currently, there is no evidence indicating the need for booster doses after completion of a primary series with HPV L1 VLP vaccines. Nevertheless, this remains an open question until such time as very long-term data on vaccine effectiveness are available (for example, more than 10 years), including in subjects who were vaccinated several years prior to sexual debut. Consequently, consideration could be given to a pre-emptive assessment of immune responses to booster doses administered to relatively small subsets at planned intervals following completion of the primary series (58). These data may be provided after initial licensure and may also give an indirect indication of priming by the primary series (see section C.8.1 below).

It is important for antibody levels to be measured at several pre-planned time points after the final vaccine dose in order to construct antibody decay curves that are sufficient to document the achievement of a plateau. These data can be collected post-licensing during long-term follow-up of antibody persistence in selected vaccinated cohorts (see section C.8.1 below).

The potential for immune interference between HPV VLP vaccines and other vaccines likely to be given concomitantly should be investigated pre- and/or post-licensing. The WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6) should be consulted regarding the design and analysis of these studies.

C.2.3 Analyses of immune responses in comparative studies

In different studies or within the same study – and depending on circumstances that limit the feasibility of some options, as discussed below – the immune response to a candidate vaccine may be compared with one or more of:
- another HPV vaccine, in which case there may be some HPV types in both vaccines (shared types) and some that are in one vaccine only (unshared types);
- the same candidate vaccine but administered at a different dose, different schedule or in a different population;
- another formulation of the candidate vaccine (for example, with and without an adjuvant or with variable numbers of HPV types);
- data obtained from a group that does not receive HPV vaccine (that is, a group that receives either a placebo or a non-HPV vaccine).

In each case, it is recommended that:

- Due to their clinical importance, comparisons of immune responses should strongly support a conclusion that vaccine efficacy against HPV types 16 and 18 is very likely to be comparable to that observed for the two initially developed HPV vaccines.
- The primary analysis population for immune responses to each HPV type is confined to those who are seronegative for the particular HPV type at baseline. Therefore, the sample-size calculations should also take into account the anticipated HPV type-specific baseline seropositivity in the population under study.
- Primary comparisons should be based on antibody titres in sera obtained at 1 month after the final dose of the intended regimen(s) unless antibody-kinetic data suggest otherwise. If a test regimen consists of a different number of doses from the control regimen (for example, 2 versus 3 doses) or if the last dose is given at a different time point (for example, at 4 months versus 6 months after the first dose) then the primary comparison should still be based on sera obtained at 1 month (or other time point based on kinetics) after the final dose, whenever that occurs. Secondary analyses should compare antibody titres measured at predefined time points from the first dose, including a comparison once antibody levels have reached a plateau.

**C.2.3.1 Comparison with a group that does not receive HPV vaccine**

Given the widespread licensing of HPV vaccines and their incorporation into routine vaccination programmes in many countries, studies of sexually active men or women that include a group that does not receive HPV vaccine will be unacceptable in many settings. Comparisons with a group that does not
receive HPV vaccine may still be possible in short-term immunogenicity studies in which subjects are considered to be at low or no risk of HPV infection (for example, prior to sexual debut) and in which all subjects eventually receive active vaccination within a protocol-specified time frame. Nevertheless, before selecting this design, the potential need for and value of comparing a candidate vaccine with a control group that does not receive HPV vaccine should be given careful consideration and should be balanced with ethical considerations regarding the withholding of a safe and effective vaccine.

Post-vaccination seropositivity rates and seroconversion rates in previously seronegative subjects have been very high with the initial HPV vaccines, and these are sensitive end-points for studies that compare vaccinated groups with groups that do not receive HPV vaccine. Consequently, these studies should aim to demonstrate superiority of seroconversion rates to each HPV type in the candidate vaccine group over the control group that does not receive HPV vaccine. The predefined criteria for concluding superiority should take into account the type-specific seroconversion rates that have been observed for the initial HPV vaccines for which efficacy was demonstrated on the basis of histological and virological data (59, 60).

The same approach is applicable whenever comparing immune responses to HPV types that are included in a candidate HPV vaccine but are not in the control HPV vaccine (that is, unshared types) since the control group is not vaccinated with respect to these types.

C.2.3.2 Comparisons between vaccinated groups

Due to the expectation that post-vaccination seropositivity rates and seroconversion rates will be very high in previously seronegative subjects, these are not sensitive end-points for discriminating between immune responses in vaccinated groups. Therefore GMT ratios for immune responses to each HPV type that is included in the candidate vaccine(s) and control HPV vaccine(s) (that is, shared types) are usually used for the primary comparisons between vaccinated groups. HPV type-specific seroconversion rates should be included among the secondary end-points.

As a general rule, for the purposes of establishing non-inferiority between vaccine groups based on GMT ratios for antibody to individual HPV types, it is suggested that the lower bound of the 95% confidence interval around the GMT ratio (test versus reference vaccine) should not fall below 0.67. Under certain circumstances, NRAs may consider allowing a lower bound of 0.5. In future, especially if an ICP can be identified or if a sponsor is able to offer a sound rationale, it may be appropriate to reconsider these acceptance criteria. In addition, any marked separations between the reverse cumulative distributions should be discussed in terms of the potential clinical implications, even if these occur only at the lower or upper ends of the curves.
Annex 4

C.2.4 **Immunobridging of vaccine efficacy**

Although there is no ICP for HPV vaccines, it is sometimes appropriate or necessary to use immunobridging to evaluate efficacy against anogenital carcinomas, preneoplastic lesions and genital warts.

Immunobridging involves a demonstration of comparable immune responses between a candidate vaccine and a licensed vaccine that has been selected in accordance with the recommendations made below in section C.2.4.1. In this way:

- the HPV type-specific efficacy observed with one HPV vaccine may be bridged to an alternative schedule (for example, reducing the number of doses or delaying the final dose) or population (for example, children prior to sexual debut in whom efficacy cannot be assessed), or to an extended version of that same vaccine (that is, with added HPV types);
- the efficacy observed with a specific licensed HPV vaccine may be bridged to a candidate vaccine for all HPV types that are shared between the two vaccines.

The comparison of immune responses should be made in accordance with the recommendations outlined above in section C.2.3.2. A successful demonstration of non-inferiority in each age and gender subgroup relevant to the indications approved for the control vaccine could support the same indications for the candidate vaccine – that is, including cervical, anal and vulvo-vaginal lesions. Nevertheless, when the inference of vaccine efficacy is based only on immunobridging data, individual NRAs may decide to restrict the indications of candidate vaccines compared with those of licensed comparator vaccines.

There may be situations in which the predefined non-inferiority margin is not met for one or more HPV types. Since the implications of such a finding for clinical efficacy cannot be determined, the possible effect on vaccine efficacy and effectiveness will have to be considered on a case-by-case basis, taking into account any possible merits of the candidate vaccine and/or test regimen (for example, a reduced or more convenient schedule, or use in a specific age group that facilitates incorporation into existing routine schedules) as well as the relative importance of the HPV type(s) in question and/or their related cross-reactive types prevalent in an individual NRA’s jurisdiction.

If non-inferiority is not demonstrated for one or more HPV types based on immune responses determined shortly after the last dose, a predefined analysis that demonstrates non-inferiority at later time points might be considered as an alternative – for example, a comparison at 18–30 months after the last vaccine dose, when current experience suggests onset of a plateau effect on the antibody decay curves. However, as time progresses since the last vaccine dose, the
difference between GMTs is likely to diminish, reducing the analytical sensitivity. In addition, basing the comparison on antibody levels at 18–30 months after the last dose would considerably delay the initial licensure of a vaccine.

C.2.4.1 Selection of the comparator vaccine

It is crucially important that immunobridging approaches to assess potential vaccine efficacy are based on appropriate comparator vaccines. In principle, the comparator vaccine should have been licensed on the basis of histological or, at least, virological primary end-points. However, in some regulatory jurisdictions, it may not be possible to license a candidate vaccine on the basis of immunobridging to the efficacy demonstrated by another HPV vaccine within a specific number of years after the comparator was first licensed. Therefore the selection of comparator vaccine(s) should be discussed with the relevant NRAs of the countries in which the candidate vaccine is intended for use. In most instances it will be appropriate to compare the candidate vaccine with a licensed vaccine that contains the same HPV types or as many shared HPV types as possible.

In future it may be that immune responses to a candidate vaccine cannot be directly compared with those to a vaccine that was previously shown to have efficacy against disease-related end-points because it is no longer marketed. For example, the original version of a vaccine that was licensed on the basis of histological and/or virological data may have been replaced by a modified version (for example, with additional HPV types). Using the modified version in immunobridging studies becomes a problem if it does not elicit very similar immune responses to the HPV types in the original version. As a result, there is a risk that modified versions could be less immunogenic and potentially less protective than the original vaccines that were developed. This inescapable fact highlights the vital importance of post-licensure documentation of vaccine effectiveness (see section C.8.2 below).

C.2.4.2 Immunobridging in specific circumstances

For the first two HPV vaccines to be developed, immunobridging was used to support their use in children aged 9–15 years. Some NRAs have subsequently accepted immunobridging to support a reduction from 3 to 2 doses in specific age subsets (55, 61–69). In each of these cases, licensure was based on demonstrating non-inferiority of immune responses in children to those in female vaccinees in the age range within which efficacy had been demonstrated following a 3-dose schedule.

In these bridging studies, the immune responses to HPV types 16 and 18 were higher in boys than in girls aged 9–15 years (64, 70). In contrast,
immune responses to some HPV types have been lower in men than in women of comparable ages, and lower in women aged 26–45 years compared to women aged 15–26 years (71, 72). Therefore, whenever an immunobridging approach is used, it is relevant to consider possible issues of interpretation based on age range and gender (see section C.5 below).

An immunobridging approach to support use in immunosuppressed populations is complicated by the likelihood of observing lower immune responses compared to those in healthy subjects (73–75). The implications of lower immune responses for vaccine efficacy are uncertain, as an ICP has not been established. NRAs will have to consider whether the anticipation of some degree of benefit in immunosuppressed populations, even if potentially lower than in immunocompetent subjects, is sufficient to support a favourable risk–benefit conclusion.

C.3 Virological data
The reliable determination of HPV infection and viral persistence at anogenital sites requires the use of carefully controlled and standardized methods of sample collection, processing and virus detection.

C.3.1 Sampling
Because HPV is cell associated, samples must contain cellular material and separate samples must be obtained from each specific anatomical site of interest.

Methods of sample collection that have been validated in large-scale epidemiological studies are recommended. The specific method used (for example, in terms of number of turns and depth of insertion of the device) should be standardized and adhered to for each study. Ideally, no changes to the method should be made during each study or during the entire clinical development programme. If changes are unavoidable, there should be adequate cross-validation to support the pooling of results obtained with different methods. The collection medium will influence the volume of sample to be extracted as well as the method of extraction. Water or collection-medium blanks should be processed and tested along with samples to ensure that no cross-contamination occurs during processing (24).

The standard approach for monitoring HPV in the cervix is for samples to be collected from the ecto-cervix and endo-cervix by clinicians after visualization of the cervix using speculum examination (24). Although a range of collection devices may be used, they should target the cervical transformation zone and each device should be compatible with the selected collection medium. Alternative methods (such as sampling only from either the ecto-cervix or the endo-cervix, or self-sampling by study participants) may be considered if appropriate validation is provided.
C.3.2 Virus detection

The assays used for the determination of virological end-points in studies intended to support licensure should provide type-specific results with high sensitivity and specificity, and should include an internal control to monitor the adequacy of samples (76, 77). The internal control, directed to a cellular DNA sequence, establishes a lower limit for acceptable cell number but will not evaluate the quality of the cellular sample. For instance, it will not distinguish between inflammatory cells (unintended sample) and epithelial cells (intended sample).

Participating in proficiency tests, when available, and obtaining satisfactory results is one step towards ensuring the quality of the assay (77). It should be noted that proficiency tests that use purified DNA will not monitor extraction efficiency, impact of possible PCR inhibitors in samples or competition between types with large differences in copy number. The WHO Human papillomavirus laboratory manual (24) provides guidance on quality assurance and quality control aspects of HPV DNA testing to laboratories performing testing for virological end-points to help ensure stability and standardization of results over time.

C.3.3 Viral persistence

As discussed above under General considerations, the vaccine efficacy studies that were conducted with the first HPV vaccines in sexually active young women demonstrated a close correlation between the primary histological end-point of high-grade cervical disease and 6-month persistent infection of the cervix with HPV (3, 78–82). On this basis, and taking into account the similarity of disease pathogenesis and progression at cervical and anal sites, viral persistence based on at least two consecutive samples obtained over at least 6 months is an acceptable alternative to histological end-points at these anatomical sites (3). Nevertheless, HPV type-specific viral persistence is a feasible primary end-point only when rates of persistent infections in vaccinees who were HPV DNA negative at study baseline for the types in question are sufficiently high to allow for studies of reasonable size. This is not expected to be a feasible end-point for studies in populations in which HPV vaccines have been introduced into routine immunization programmes and in which there has been a very high uptake. It is also not likely to be a feasible end-point for HPV types that are rarely encountered, regardless of any vaccine usage.

The demonstration of viral persistence should be based on consecutive type-specific HPV DNA positive samples obtained from the same anatomical site over at least 6 months from the time of the first positive result. Because the timing of incident infections after completion of the vaccination series cannot be predicted, an event-driven analysis is often employed. Thus, the primary analysis is conducted when a protocol-defined number of total cases of viral persistence
(which may be based on HPV types 16 and 18 or otherwise defined by types) has been accumulated. This total number should be estimated to provide sufficient statistical power to detect meaningful differences between treatment groups. In the primary analysis, the cases of viral persistence should be counted from a predefined period (for example, at least 2 weeks) after the final dose of the vaccination series. A secondary analysis could be based on counting all cases from the time of the first dose. Protocols should address how to handle sequential results when at least one of a series of samples is negative for HPV DNA but is followed by one or more positive samples.

C.3.3.1 Comparison with a group that does not receive HPV vaccine

For the same reasons discussed above in section C.2.3.1, studies of viral persistence that include a group that does not receive HPV vaccine will be unacceptable in many settings. If such a design is still considered acceptable and if incidence rates are sufficiently high to make the study feasible (that is, due to lack of widespread implementation of HPV vaccination in the regions where the study will be conducted) then a superiority design could be used.

Depending on what is known about incidence rates, there could be co-primary end-points of viral persistence for each of HPV types 16 and 18 or a single composite primary end-point based on viral persistence for both types. For candidate vaccines containing additional HPV types, a primary analysis could be based on pooled data for HPV types 16 and 18 with a co-primary or secondary analysis based on viral-persistence data pooled for all other HPV types and supportive analyses of viral persistence for each HPV type. Separate or combined studies addressing cervical and anal sites and/or by gender could be considered.

C.3.3.2 Comparisons between HPV vaccines

As discussed above, it is much more likely that another HPV vaccine containing types 16 and 18, with or without additional types, would have to be administered to the control group. Assuming that the candidate vaccine has good efficacy, experience obtained with the first two HPV vaccines indicates that few, if any, cases of viral persistence of any HPV type will occur in any reasonable time frame, and it may be necessary to resort to an immunobridging approach for assessment of vaccine efficacy. Nevertheless, whenever viral persistence is assessed among the study end-points, the following approaches are suggested:

- Although persistent infection due to HPV types 16 or 18 is not likely to be a feasible primary end-point, all cases should be monitored in case the candidate vaccine has unexpectedly low efficacy (for example, there could be a negative impact of additional HPV types on the protection against HPV types 16 and 18 afforded by a highly multivalent candidate vaccine).
For any additional HPV types shared between the candidate and comparator vaccines, supportive analyses should compare viral-persistence data pooled across all shared types as well as for each individual type.

For any HPV types in the candidate vaccine only, viral persistence may be pooled across the additional types, but supportive analyses should be conducted for individual types.

C.4 Histological data

As discussed above in sections C.2 and C.3:

- Viral-persistence end-points can be used instead of histological end-points to support the efficacy of a candidate vaccine.
- The acceptability of a control group that does not receive HPV vaccine is rapidly diminishing.
- The feasibility of using histological end-points is influenced by the same issues that affect viral-persistence end-points – that is, decreasing rates of events due to widespread vaccine use and very low rates of lesions associated with some HPV types regardless of any vaccine usage.

If histological data are to be collected in a study, it is recommended that the end-points used in the pivotal efficacy studies that were conducted with the two initial HPV vaccines in women and with the one in men should be used – that is, incident high-grade anogenital lesions associated with positivity for oncogenic HPV types (59, 60, 83).

C.5 Evaluation of vaccine efficacy in different settings

This section summarizes approaches to assessing potential vaccine efficacy against anogenital carcinomas in different settings. The following issues should be noted:

- Depending on the local circumstances (that is, whether any HPV vaccines have been licensed, the types of HPV L1 VLP they contain and whether they have been incorporated into routine vaccination programmes), individual NRAs may have alternative requirements to the recommendations made in this section.
- For reasons discussed above in section C.4, histological end-points are not considered.
- In all settings, only serological data can be obtained from subjects prior to sexual debut.
This section does not cover the assessment of efficacy against genital warts. For a candidate HPV vaccine containing types 6 and 11, the considerations regarding whether genital warts is a feasible end-point are the same as those outlined in section C.3.3.1 for viral persistence as an end-point. In all other settings, efficacy against genital warts will have to be based on demonstrating similar immune responses to these two HPV types between the candidate vaccine and a suitable control vaccine, as outlined in section C.2.4.

C.5.1 Efficacy against HPV types 16 and 18
Efficacy may be evaluated by one of the following approaches:

- viral persistence under the circumstances described in section C.3.3.1
- immunobridging to a licensed vaccine, as described in section C.2.4.

C.5.2 Efficacy against other HPV types
An evaluation of efficacy against other HPV types associated with anogenital carcinomas is necessary in the following situations:

- Addition of one or more HPV type(s) associated with anogenital carcinomas to a licensed vaccine that contains at least HPV types 16 and 18 L1 VLPs. In this case, comparisons should be made with the original licensed vaccine and/or another licensed vaccine that contains as many shared types as possible.
- De novo development of a vaccine that contains HPV types 16 and 18 plus additional types, in which case all the additional types may already be included in a licensed vaccine, or one or more of the additional types may not be present in any licensed vaccine. Comparisons could be made with one or more of the following: (a) a group that does not receive HPV vaccine, if this option is still appropriate; (b) a licensed vaccine that contains HPV types 16 and 18 but does not include the additional types that are in the candidate vaccine; (c) a licensed vaccine that contains as many shared types as possible.

C.5.2.1 Shared HPV types
Efficacy may be evaluated by one of the following approaches:

- viral persistence, as described above in section C.3.3.2;
- demonstration of non-inferiority of immune responses to HPV types shared between the candidate and comparator vaccines, as described above in section C.2.3.2.
C.5.2.2 Unshared HPV types

- For HPV types in the candidate vaccine but not in the comparator vaccine, there is the possibility of demonstrating superiority for the candidate vaccine on the basis of a composite viral-persistence endpoint (either all additional types or all types), as described above in section C.3.3.1. In reality, the incidence of viral persistence due to the unshared types may be so low that it precludes this approach because of the sample size that would be needed.

- If the approach described above is not feasible, the assessment of efficacy of the unshared types can be based only on immunological data. There are no sound interpretive criteria that can be recommended to infer vaccine efficacy. Nevertheless, one approach may be to demonstrate that the seroconversion rate to each unshared type is non-inferior to whichever is lowest of the seroconversion rates to HPV types 16 and 18 in the licensed vaccine group. It is not appropriate to base the comparisons on the GMT ratios because these are known to be very variable between types.

C.5.3 Modification(s) of the mode of use

Comparisons of immunological data, as described above in sections C.2.3 and C.2.4, may be used to support licensure in the following settings:

- changes in schedule or age range for a licensed HPV vaccine;
- changes in formulation and/or route of administration of a vaccine that includes L1 VLPs manufactured exactly as for the original version of a licensed vaccine;
- de novo development of a candidate vaccine with a different age range or schedule from that of licensed HPV vaccines, or specifically formulated for a route of administration different from that of licensed HPV vaccines.

Additional comments regarding specific situations are provided below.

C.5.3.1 Extending the age range downwards

The use of HPV vaccines from the age of 9 years was supported by immunobridging, as described above in section C.2.4.2. In the absence of an ICP that might be applied to interpret long-term antibody persistence data it has been assumed that vaccination from 9 years of age will provide protection in the years following sexual debut on the basis of observations that initial post-vaccination antibody titres fall to a plateau that is maintained after several years.
If a vaccine is proposed for use from < 9 years of age, it is recommended that the potential value of vaccination even earlier in life be discussed with NRAs. If this strategy is pursued, it is recommended that immune responses in male and female subjects in the new target age range (for example, 6 to < 9 years) are directly compared with those in men and women aged 15–26 years – that is, in the age group in which efficacy has been demonstrated on the basis of histological and virological end-points. A comparison between children aged < 9 years and those aged 9 to < 15 years should not be the basis for licensure since efficacy has not been established in the latter age range.

C.5.3.2 Extending the age range upwards

Studies using histological or viral-persistence end-points have been conducted in women aged 26–45 years but not in men aged > 26 years. Interpretation of the available data by individual NRAs has varied. As a result, different approaches have been taken to the prescribing information in terms of whether to impose an upper age limit or to omit any upper age limit for one or both sexes.

It is not expected that efficacy can be demonstrated in individuals > 26 years of age on the basis of prevention of HPV-associated advanced dysplastic lesions or cervical cancer. As the routine use of HPV vaccines increases, the feasibility of demonstrating efficacy based on viral persistence will decrease. An immunobridging approach may not succeed since immune responses may be naturally lower as age increases. As a result, it is not possible to recommend an approach to support licensure of HPV vaccines for use in subjects older than 26 years. NRAs must decide on a case-by-case basis whether additional data would be needed to include older subjects in the indications for use, taking into account what seems feasible and the public health considerations applicable to the region.

C.6 Cross-protection

The sponsor may choose to assess the ability of a vaccine to elicit cross-reacting neutralizing antibody against non-vaccine HPV types that are closely related to the types included in a vaccine. However, experience indicates that these data cannot be used to establish the ability of a vaccine to confer cross-protection (84).

Thus far, claims for cross-protection against related HPV types not included in a vaccine have been based on relatively short-term histological and viral-persistence data. Since the assessment of specific epitopes that elicit cross-protection is not part of the HPV L1 VLP vaccine specifications, the degree of cross-protection may be very vaccine specific. Currently, or in the future, it may not be feasible to demonstrate cross-protection based on virological persistence. Reliance on immunobridging to a licensed vaccine is not straightforward because:
available data suggest that cross-neutralizing antibody may not be predictive for cross-protection;

there would have to be a case-by-case evaluation of the validity of extrapolating cross-protection claims between products based on the possibility that small differences between epitopes could have an impact on cross-protection.

Therefore, if viral persistence cannot be used as an end-point, it does not appear that future cross-protection claims can be supported.

**C.7 Safety**

The general considerations for the pre-licensure assessment of safety during clinical studies are those outlined in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6).

In the specific case of HPV vaccines, it is important that the safety database adequately covers the entire target age range and both sexes, as applicable to the authorized indications. However, it is not necessary to generate sufficient safety data to estimate frequencies of uncommon adverse events in each subset. The numbers vaccinated within each sex and age subgroup should be supported by discussion of any anticipated differences that could preclude assumptions of similar safety profiles according to the characteristics of vaccinees (for example, if the reactogenicity profile seems to be very different between men and women or between adults and younger subjects).

Regardless of protocol recommendations for studies conducted among sexually active women, numerous pregnancies have been documented in vaccinees. Every effort should be made to estimate the stage of gestation in relation to vaccine doses and to document the outcome of the pregnancy. Specific studies in pregnant women are not recommended at this time (85).

Assessment of safety in the post-licensure period is discussed below in section C.8.3.

**C.8 Post-licensing evaluation**

**C.8.1 Immunological follow-up**

The duration of immunological follow-up to document antibody persistence at the time of initial licensure may be very limited. At the time of licensure, plans should be in place to document antibody persistence to each HPV type in the vaccine in the longer term. These data should be obtained from subsets of subjects who were initially vaccinated across the entire age range of use, and for both sexes. The final duration of follow-up should be agreed with NRAs on the basis of knowledge of antibody decay curves and of the magnitude of the initial immune response to vaccination (see section C.2.2 above).
As discussed in section C.2.2, since it is not yet known whether additional doses of vaccine may be needed after completion of the primary series to assure prolonged protection, an assessment of immune responses to booster doses at planned intervals following completion of the primary series should be conducted (58).

C.8.2 Vaccine effectiveness and related issues

It is important to obtain data on vaccine effectiveness and the risk of an HPV type-replacement phenomenon in the post-licensing period. Some NRAs consider that collection of these data is a responsibility of the licence holder. Nevertheless, the generation of reliable data usually requires activation of large-scale public health initiatives and disease monitoring on a national or regional basis (for example, the use of cancer registries to capture the effect of HPV vaccines on rates of HPV-related anogenital carcinomas). Thus, the post-licensing commitments that individual NRAs may require from licence holders are expected to relate to cooperative efforts with appropriate bodies (for example, public–private partnerships, where these are deemed to be acceptable).

In some regions where routine vaccination against HPV is not yet established there may be specific oncogenic HPV types that are detected relatively frequently (with or without HPV types 16/18 or other types) in association with pre-cancerous anogenital lesions and anogenital carcinomas. Nevertheless, for HPV vaccines that contain the specific type(s) in question, pre-licensure estimates of vaccine efficacy based on histological or viral-persistence end-points may not be available. In such regions, especially when claims for efficacy against these types are based on immunological data only, the responsible NRAs may consider it particularly important to obtain vaccine-effectiveness data.

If a licensed vaccine has been granted claims for cross-protection based on relatively short-term histological and viral-persistence data (see section C.6 above), there should be further follow-up to confirm these claims in the post-licensure period.

C.8.3 Safety monitoring

The general considerations for safety surveillance and for the development of a pharmacovigilance plan are the same as for all other types of vaccine (6).

Where HPV vaccines have been introduced into routine vaccination programmes they are mostly administered to children prior to sexual debut. However, initial catch-up programmes that include older subjects have sometimes resulted in inadvertent vaccination during early pregnancy. Safety surveillance programmes should capture the outcomes of these pregnancies (85).
There may be some instances in which NRAs require specific post-licensing safety studies to be conducted by the licence holder in order to address particular concerns. The design and time frame for reporting these studies should be agreed at the time of initial licensure.

**Part D. Recommendations for NRAs**

**D.1 General recommendations**

The general recommendations for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (86) and Guidelines for independent lot release of vaccines by regulatory authorities (87) should apply. These recommendations specify that no new biological substance should be released until consistency of batch manufacturing and quality has been demonstrated.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines, should be discussed with and approved by the NRA.

For control purposes, the relevant International Standards currently in force should be obtained for the purpose of calibrating national, regional and working standards (46). The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release.

Consistency of production has been recognized as an essential component in the quality assurance of recombinant human papillomavirus virus-like particle vaccines. In particular, the NRA should carefully monitor production records and quality control test results for clinical lots, as well as for a series of consecutive lots of the vaccine.

**D.2 Official release and certification**

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations (87).

A protocol for the manufacturing and control of recombinant human papillomavirus virus-like particle vaccines, based on the model protocol provided in Appendix 1, and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for the release of a vaccine for use.

A lot release certificate signed by the appropriate NRA official should then be provided if requested by a manufacturing establishment, and should certify whether or not the lot of vaccine in question meets all national requirements and/or Part A of these WHO Recommendations. The certificate
should provide sufficient information on the vaccine lot. The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided in Appendix 2.

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Further changes were subsequently made to document WHO/BS/2015.2252 by the WHO Expert Committee on Biological Standardization.
References


Appendix 1

Model protocol for the manufacturing and control of recombinant human papillomavirus virus-like particle vaccines

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by the manufacturer to the NRA. Information and tests may be added or omitted as necessary with the approval of the NRA.

It is possible that a protocol for a specific product may differ in detail from the model provided here. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO Recommendations for a particular product should be provided in the protocol submitted.

The section concerning the final lot must be accompanied by a sample of the label and a copy of the leaflet (package insert) that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a lot release certificate from the NRA or from the NCL in the country in which the vaccine was produced or released, stating that the product meets national requirements as well as the recommendations in Part A of this document.

Summary information on the final lot

International name: ____________________________
Trade name/commercial name: ____________________________
Product licence (marketing authorization) number: ____________________________
Country: ____________________________
Name and address of manufacturer: ____________________________
Name and address of licence holder, if different: ____________________________

Final lot

Batch number(s)

Final lot: ____________________________
Final bulk: ____________________________
Type of container: ____________________________
Total number of filled containers in this final lot: ____________________________
Number of doses per container: ________________________________
Antigen concentration/volume of single human dose: __________________

**Production information**

Batch number of each monovalent bulk (purified and/or adsorbed): ________________________________
Site of manufacture of each monovalent bulk: ________________________________
Date of manufacture of each monovalent bulk: ________________________________
Site of manufacture of adjuvant(s): ________________________________
Date of manufacture of adjuvant(s): ________________________________
Site of manufacture of final bulk: ________________________________
Date of manufacture of final bulk: ________________________________
Site of manufacture of final lot: ________________________________
Date of manufacture of final lot: ________________________________
Date on which last determination of potency was started or date of start of period of validity: ________________________________
Shelf-life approved (months): ________________________________
Expiry date: ________________________________
Storage conditions: ________________________________
Release date: ________________________________

*A genealogy of the lot numbers of all vaccine components used in the formulation of the final lot will be informative.*

The following sections are intended for the reporting of the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. Therefore, if any test has to be repeated this must be indicated. Any abnormal result must be recorded on a separate sheet.

**Starting materials**

*The information requested below is to be presented on each submission. Full details on master and working seed lots, and cell banks are requested upon first submission only and whenever a change has been introduced.*

**Control of source materials (section A.3)**

**Cell cultures for antigen production (section A.3.1)**

Source of HPV antigen (expression system): ________________________________
Master cell bank (MCB) lot number and preparation date: ________________________________
Population doubling level (PDL) of MCB: ____________________________
Date of approval of protocols indicating compliance with
the requirements of the relevant monographs and
with the product licence: ____________________________

Manufacturer’s working cell bank (WCB) lot number
and preparation date: ____________________________
Population doubling level (PDL) of manufacturer’s WCB: ______________
Date of approval of protocols indicating compliance with
the requirements of the relevant monographs and with
the product licence: ____________________________
Production cell lot number: ____________________________

Identification of cell substrate
Method: ____________________________
Specification: ____________________________
Date: ____________________________
Result: ____________________________

Nature and concentration of antibiotics or selecting agent(s) used
in production cell culture maintenance medium:

Identification and source of starting materials used in preparing
production cells, including excipients and preservatives
(particularly any materials of human or animal origin such as
albumin or serum): ____________________________

Virus seed lots if a recombinant baculovirus
expression vector is used (section A.3.2)
Sufficient detail should be provided on inoculum intermediates, including the
passage level from the master seed and the length and conditions of storage, if any.

Virus strain and reference number used to prepare the
licensed HPV vaccine: ____________________________
Master seed lot number and preparation date: ____________________________
Number of passages between two seeds mentioned above: ____________________________
Date of approval of protocols indicating compliance with
the requirements of the relevant monographs and
with the product licence: ____________________________
Working seed lot number and
preparation date: ____________________________
Passage level from master seed lot: ____________________________

Date of approval of protocols indicating compliance with the requirements of the relevant monographs and with the product licence: ____________________________

Each seed lot should be tested for the following:

**Identity**
- Method: ____________________________
- Specification: ____________________________
- Date: ____________________________
- Result: ____________________________

**Bacteria and fungi**
- Method: ____________________________
- Media: ____________________________
- Volume inoculated: ____________________________
- Date of start of test: ____________________________
- Date of end of test: ____________________________
- Result: ____________________________

**Mycoplasmas, spiroplasma, entomoplasma and mesoplasma**
- Method: ____________________________
- Volume inoculated: ____________________________
- Date of start of test: ____________________________
- Date of end of test: ____________________________
- Result: ____________________________

**Adventitious agents**
- Method: ____________________________
- Specification: ____________________________
- Date: ____________________________
- Result: ____________________________

**Mycobacterium spp. (if applicable)**
- Method: ____________________________
- Media: ____________________________
- Volume inoculated: ____________________________
- Date of start of test: ____________________________
- Date of end of test: ____________________________
- Result: ____________________________
Recombinant baculovirus concentration

Method: 

Specification: 

Date: 

Result: 

Control cell cultures if mammalian or insect cells are used for production and recombinant viral vector cannot be neutralized, thus interfering with testing.

Provide information on control cells corresponding to each single harvest (section A.3.2.1.5).

Ratio or proportion of control to production cell cultures: 

Volume of control cells: 

Dates of observation of cultures: 

Percentage rejected for nonspecific reasons: 

Result: 

Karyotype

Method: 

Probe: 

Reference cells: 

Date of start of test: 

Date of end of test: 

Result: 

Identity test by DNA fingerprinting (if applicable)

Method: 

Probe: 

Reference cells: 

Restriction enzymes: 

Date of start of test: 

Date of end of test: 

Result: 

Haemadsorbing viruses

Type(s) of red blood cell (RBC): 

Storage time and temperature of RBC: 

Incubation time and temperature of RBC:
Percentage cultures tested: ________________________________
Date of start of test: ________________________________
Date of end of test: ________________________________
Result: ________________________________

Tests on supernatant fluids or cell lysates for other adventitious agents (if relevant)
Date of sampling from production cell cultures: ________________

**Type of simian cells**
Quantity of sample inoculated: ________________________________
Incubation temperature: ________________________________
Date of start of test: ________________________________
Date of end of test: ________________________________
Percentage of viable culture at the end: ________________________________
Result: ________________________________

**Type of human cells**
Quantity of sample inoculated: ________________________________
Incubation temperature: ________________________________
Date of start of test: ________________________________
Date of end of test: ________________________________
Percentage of viable culture at the end: ________________________________
Result: ________________________________

**Type(s) of other cells**
Quantity of sample inoculated: ________________________________
Incubation temperature: ________________________________
Date of start of test: ________________________________
Date of end of test: ________________________________
Percentage of viable culture at the end: ________________________________
Result: ________________________________

**Bacteria and fungi**
Method: ________________________________
Media: ________________________________
Volume inoculated: ________________________________
Date of start of test: ________________________________
Date of end of test: ________________________________
Result: ________________________________
**Mycoplasmas**

- **Method:**
- **Volume inoculated:**
- **Date of start of test:**
- **Date of end of test:**
- **Result:**

---

**Single antigen harvests (or pools) (section A.4)**

- **Batch number(s):**
- **Date of inoculation:**
- **Date of harvesting:**
- **Volume(s) of cell suspension/paste or supernatant,**
  storage temperature, storage time and
  approved storage period:

---

**Culture purity or sterility for bacteria and fungi**

- **Method:**
- **Media:**
- **Volume inoculated:**
- **Date of start of test:**
- **Date of end of test:**
- **Result:**

---

**Identity of HPV type**

- **Method:**
- **Specification:**
- **Date:**
- **Result:**

---

**Consistency of yield (for example, infectivity of replicating vector virus and/or HPV antigen concentration, if applicable)**

- **Method:**
- **Reference preparation:**
- **Specification:**
- **Date:**
- **Result:**
In addition, information on the following tests should be provided if mammalian cells or insect cells are used:

**Adventitious agents**
- Method: _____________________________
- Specification: _____________________________
- Date: _____________________________
- Result: _____________________________

**Mycoplasmas**
- Method: _____________________________
- Volume inoculated: _____________________________
- Date of start of test: _____________________________
- Date of end of test: _____________________________
- Result: _____________________________

**Purified monovalent antigen bulk (section A.5)**
- Batch number(s) of purified bulk: _____________________________
- Date(s) of purification(s): _____________________________
- Volume(s), storage temperature, storage time and approved storage period: _____________________________

**Identity (if applicable)**
- Method: _____________________________
- Specification: _____________________________
- Date: _____________________________
- Result: _____________________________

**Composition (protein, lipid, polysaccharide, if applicable)**
- Method: _____________________________
- Specification: _____________________________
- Date: _____________________________
- Result: _____________________________

**Protein purity (add PAGE photographs)**
- Method: _____________________________
- Specification: _____________________________
- Date: _____________________________
- Result: _____________________________
Protein content
Method: ________________________________
Specification: ________________________________
Date: ________________________________
Result: ________________________________

Antigen content (if applicable)
Method: ________________________________
Specification: ________________________________
Date: ________________________________
Result: ________________________________

Ratio of antigen:protein content (if applicable)
Specification: ________________________________
Result: ________________________________

Bacteria and fungi
Method: ________________________________
Media: ________________________________
Volume inoculated: ________________________________
Date of start of test: ________________________________
Date of end of test: ________________________________
Result: ________________________________

Percentage intact L1 monomer
Method: ________________________________
Specification: ________________________________
Date: ________________________________
Result: ________________________________

VLP size and structure
Report on this is needed until production consistency is demonstrated
Method: ________________________________
Specification: ________________________________
Date: ________________________________
Result: ________________________________

Tests for reagents used during purification and other phases of manufacture (if relevant)
Method: ________________________________
Specification: ________________________________
Date: __________________________________________________________
Result: _______________________________________________________

**Residual DNA (if applicable)**
- Method: _______________________________________________________
- Specification: _________________________________________________
- Date: _________________________________________________________
- Result: _______________________________________________________

**Bovine serum albumin content (if mammalian or insect cells and animal serum are used for production).**
- Method: _______________________________________________________
- Specification: _________________________________________________
- Date: _________________________________________________________
- Result: _______________________________________________________

**Viral clearance**
*This is performed during vaccine manufacturing development and/or process validation and is not intended for batch release (see section A.5.1.11).*
- Method: _______________________________________________________
- Specification: _________________________________________________
- Date: _________________________________________________________

**Adsorbed monovalent antigen bulk (section A.6)**
- Batch number(s) of adsorbed monovalent antigen bulk: __________
- Adsorption date: ______________________________________________
- Batch number(s) of all components used during adjuvant adsorption:
  _____________________________________________________________
- Volume, storage temperature, storage time and approved storage period: ______________________________
  _____________________________________________________________

**Bacteria and fungi**
- Method: _______________________________________________________
- Media: ________________________________________________________
- Volume inoculated: ____________________________________________
- Date of start of test: __________________________________________
- Date of end of test: ___________________________________________
Bacterial endotoxins
Method: __________________________________________
Specification: ______________________________________
Date: ____________________________________________
Result: __________________________________________

Identity
Method: __________________________________________
Specification: ______________________________________
Date: ____________________________________________
Result: __________________________________________

Adjuvant concentration (if relevant)
Method: __________________________________________
Specification: ______________________________________
Date: ____________________________________________
Result: __________________________________________

Degree of adsorption (if applicable)
Method: __________________________________________
Specification: ______________________________________
Date: ____________________________________________
Result: __________________________________________

pH
Method: __________________________________________
Specification: ______________________________________
Date: ____________________________________________
Result: __________________________________________

Antigen content (if applicable)
Method: __________________________________________
Batch number of reference vaccine and assigned potency: _______________
Specification: ______________________________________
Date: ____________________________________________
Result: __________________________________________

Final bulk (section A.7)
Batch number: ______________________________________
Date of manufacture: ___________________
Batch numbers and volumes of adsorbed bulk vaccines used for the formulation of the final bulk vaccine: ______________________

Batch number(s) and volume(s) of bulk alum diluent: ______________________

Volume, storage temperature, storage time and approved storage period: ______________________

**Bacteria and fungi**

Method: ______________________

Media: ______________________

Volume inoculated: ______________________

Date of start of test: ______________________

Date of end of test: ______________________

Result: ______________________

**Adjuvants**

Method: ______________________

Specification: ______________________

Date: ______________________

Result: ______________________

**Degree of adsorption (if applicable)**

Method: ______________________

Specification: ______________________

Date: ______________________

Result: ______________________

**Preservatives (if applicable)**

Method: ______________________

Specification: ______________________

Date: ______________________

Result: ______________________

**Potency**

*If an in vitro assay of each type is used*

Method: ______________________

Batch number of reference vaccine and assigned potency: ______________________

Specification: ______________________

Date: ______________________

Result: ______________________
If an in vivo assay is used

Species, strain, sex and weight specifications: ____________________________

Dates of vaccination, bleeding: ____________________________

Date of assay of each type: ____________________________

Batch number of reference vaccine and assigned potency: ____________________________

Vaccine doses (dilutions) and number of animals responding at each dose for each type: ____________________________

ED_{50} of reference and test vaccine for each type: ____________________________

Potency of test vaccine versus reference vaccine for each type with 95% confidence limits of mean: ____________________________

Validity criteria for each type: ____________________________

Osmolality (if applicable)

Method: ____________________________

Specification: ____________________________

Date: ____________________________

Result: ____________________________

Freezing point (if applicable)

Method: ____________________________

Specification: ____________________________

Date: ____________________________

Result: ____________________________

Final lot (section A.9)

Batch number: ____________________________

Date of filling: ____________________________

Type of container: ____________________________

Filling volume: ____________________________

Number of containers after inspection: ____________________________

Appearance

Method: ____________________________

Specification: ____________________________
Date: ______________________________________
Result: ______________________________________

**Identity (each type)**
Method: ______________________________________
Specification: ______________________________________
Date: ______________________________________
Result: ______________________________________

**Bacteria and fungi**
Method: ______________________________________
Media: ______________________________________
Volume inoculated: ______________________________________
Date of start of test: ______________________________________
Date of end of test: ______________________________________
Result: ______________________________________

**pH**
Method: ______________________________________
Specification: ______________________________________
Date: ______________________________________
Result: ______________________________________

**Osmolality (if applicable)**
Method: ______________________________________
Specification: ______________________________________
Date: ______________________________________
Result: ______________________________________

**Freezing point (if applicable)**
Method: ______________________________________
Specification: ______________________________________
Date: ______________________________________
Result: ______________________________________

**Preservatives (if applicable)**
Method: ______________________________________
Specification: ______________________________________
Date: ______________________________________
Result: ______________________________________
**Pyrogenic substances**

<table>
<thead>
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<th>Method:</th>
<th>Specification:</th>
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<tbody>
<tr>
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<td>Result:</td>
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</table>

<table>
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<th>Adjuvant content</th>
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<td>Date:</td>
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<tr>
<th>Protein content (or calculated value)</th>
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<tr>
<td>Method:</td>
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<td>Date:</td>
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</table>

<table>
<thead>
<tr>
<th>Degree of adsorption of each type (if applicable)</th>
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<tbody>
<tr>
<td>Method:</td>
</tr>
<tr>
<td>Date:</td>
</tr>
</tbody>
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<tr>
<th>Potency</th>
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</thead>
<tbody>
<tr>
<td>If an in vitro assay of each type is used</td>
</tr>
<tr>
<td>Method:</td>
</tr>
<tr>
<td>Specification:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>If an in vivo assay is used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species, strain, sex and weight specifications:</td>
</tr>
<tr>
<td>Dates of vaccination, bleeding:</td>
</tr>
<tr>
<td>Date of assay of each type:</td>
</tr>
<tr>
<td>Batch number of reference vaccine and assigned potency:</td>
</tr>
</tbody>
</table>
Vaccine doses (dilutions) and number of animals responding at each dose for each type: ________________________

ED$_{50}$ of reference and test vaccine for each type: ________________________

Potency of test vaccine versus reference vaccine for each type with 95% fiducial limits of mean: ________________________

Validity criteria for each type: ________________________

Date of start of period of validity: ________________________

**General safety (unless omission authorized)**

Method: ________________________

Specification: ________________________

Date: ________________________

Result: ________________________

**Certification by the manufacturer**

Name of Head of Quality Control (typed) ________________________

Certification by the person from the control laboratory of the manufacturing company taking overall responsibility for the production and quality control of the vaccine.

I certify that lot no. ________________________ of recombinant human papillomavirus virus-like particle vaccine, whose number appears on the label of the final containers, meets all national requirements and/or satisfies Part A$^1$ of the 2015 WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines.$^2$

Signature ________________________

Name (typed) ________________________

Date ________________________

**Certification by the NRA**

If the vaccine is to be exported, attach the NRA Lot Release Certificate (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.

---

$^1$ With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

Appendix 2

Model NRA Lot Release Certificate for recombinant human papillomavirus virus-like particle vaccines

Certificate No. _______________________

The following lot(s) of recombinant human papillomavirus virus-like particle vaccine produced by ___________________________ in __________________________, whose numbers appear on the labels of the final containers, meet all national requirements\(^3\) and Part A\(^4\) of the 2015 WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines\(^5\) and comply with WHO good manufacturing practices for pharmaceutical products: main principles;\(^6\) WHO good manufacturing practices for biological products;\(^7\) and Guidelines for independent lot release of vaccines by regulatory authorities.\(^8\)

The release decision is based on __________________________.

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);

\(^1\) Name of manufacturer.
\(^2\) Country of origin.
\(^3\) If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA.
\(^4\) With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.
\(^9\) Evaluation of the summary protocol, independent laboratory testing and/or procedures specified in a defined document etc., as appropriate.
- type of container used;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and expiry date;
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other appropriate authority)

Name (typed) ________________________________
Signature ________________________________
Date ________________________________
Annex 5

Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions

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Appendix  Product-specific ECTC evaluation of a model monovalent polysaccharide conjugate vaccine 257
Guidelines published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CTC</td>
<td>controlled temperature chain</td>
</tr>
<tr>
<td>ECTC</td>
<td>extended controlled temperature conditions</td>
</tr>
<tr>
<td>EU</td>
<td>ELISA Unit(s)</td>
</tr>
<tr>
<td>IFPMA</td>
<td>International Federation of Pharmaceutical Manufacturers &amp; Associations</td>
</tr>
<tr>
<td>LB</td>
<td>lower bound</td>
</tr>
<tr>
<td>LL</td>
<td>lower limit</td>
</tr>
<tr>
<td>MRP</td>
<td>minimum release potency</td>
</tr>
<tr>
<td>NLT</td>
<td>not less than</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMT</td>
<td>not more than</td>
</tr>
<tr>
<td>NRA</td>
<td>national regulatory authority</td>
</tr>
<tr>
<td>PS</td>
<td>polysaccharide</td>
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</table>
1. Introduction

Vaccines are complex biological products and may undergo degradation during long-term storage under cold chain conditions (for example, 2–8 °C) and this is typically enhanced at higher temperatures. Consequently, establishing the stability characteristics of products is a critical element of the overall evaluation by a national regulatory authority (NRA) to ensure that licensed vaccines remain efficacious at the end of their shelf-life when stored under the approved conditions. In response to the stability assessment needs identified by NRAs, WHO developed guidelines on the stability evaluation of vaccines to assist its Member States (1). While it is well understood that vaccine quality depends on cold chain storage, it is also recognized that immunization programmes in certain regions face substantial challenges in maintaining cold chains in the field, especially during the final stage of distribution in remote areas (2, 3). To address these distribution challenges and expand immunization programmes into specific regions WHO developed a “controlled temperature chain” (CTC) programme. This programme currently requires that a vaccine exhibits a stability profile suitable for a single exposure to at least 40 °C for a minimum of 3 days just prior to administration, while remaining compliant with the approved vaccine specifications. Additionally, the programme requires that the CTC provision should be included in the licensure by the relevant NRA and by WHO prequalification (4).

During the development of these WHO Guidelines, the term “extended controlled temperature conditions” (ECTC) was proposed to distinguish regulatory requirements from WHO CTC programme aspects. This terminology convention is used throughout the following guidance. An ECTC assessment should assure the performance of a vaccine following short-term exposure to temperatures above those of a typical cold chain and could consider any temperature above the traditional 2–8 °C cold chain that might support vaccine distribution. Thus ECTC is independent of the specific programmatic requirements of the current WHO CTC programme. Vaccines licensed for use under ECTC are required to have sufficient information on the approved conditions (such as maximum temperature and time) on the package insert.

An example of an approved ECTC product that is also compliant with WHO CTC programme requirements is the meningitis A conjugate vaccine MenAfriVac. ECTC evaluation and subsequent label approval has made it possible to distribute this vaccine to populations that would otherwise have been difficult to immunize because of the limited availability of traditional cold chains (5, 6). ECTC labelling allows greater flexibility in vaccination campaigns by reducing the burden on health-care workers and, once the vaccine is removed from the cold chain to allow for immunization in remote areas, saving the costs of further refrigeration infrastructure and eliminating the need for wet ice.
Additionally, this on-label NRA-approved approach under the ECTC avoids off-label vaccine administration which is inconsistent with official guidance on best practice (7).

These WHO Guidelines arose from WHO immunization programme requirements (2, 3) and from the resulting discussions of international vaccine stability experts at WHO-sponsored consultations in Ottawa, Canada (8) and Langen, Germany (9). The ECTC guidance provided here is intended to supplement the broader WHO Guidelines on stability evaluation of vaccines (1) and focuses on ECTC-specific issues not covered in existing guidance with as little overlap as possible. The key elements of this document are the applications of the mathematical modelling and statistical concepts in existing stability guidance (1) and related publications (10, 11) to the unique short-term requirements that apply to some cases of vaccine distribution and use (8, 9). Early dialogue between manufacturers and regulators, as well as with public health officials in immunization programmes, is recommended so that those vaccines compatible with ECTC use can be evaluated for licensure by the appropriate NRA.

These Guidelines should be read in conjunction with the existing WHO Guidelines on stability evaluation of vaccines (1). The guidance which follows takes the form of WHO Guidelines rather than Recommendations because vaccines represent a heterogeneous class of agents and the stability testing programme will need to be adapted to suit the product in question. WHO Guidelines allow greater flexibility than Recommendations with respect to specific issues related to particular vaccines.

2. Scope

This document provides guidance to NRAs and manufacturers on the scientific and regulatory issues to be considered in evaluating the stability of vaccines for use under ECTC. Evaluation criteria are provided for the approval of short-term temperature conditions, in addition to those defined for long-term storage of a given vaccine, in situations where the vaccine is exposed to these short-term conditions immediately prior to administration.

This document does not provide guidance on the stability evaluation of vaccines that are inadvertently or repeatedly exposed to temperatures for which they were not licensed.

3. Terminology

The definitions given below apply to the terms as used in these WHO Guidelines. They may have different meanings in other contexts.
Accelerated stability studies: studies designed to determine the impact over time of exposure to temperatures higher than those recommended for storage on the physical, chemical, biological, biopharmaceutical and microbiological characteristics of a vaccine. When the accelerated temperature conditions are equivalent to or higher than the ECTC under evaluation, the accelerated stability data can be considered in support of the target ECTC.

Cold chain: a series of storage and transport links used for keeping and distributing vaccines in good condition until use according to the approved long-term storage condition and shelf-life. The typical temperature for the long-term storage condition is 2–8 °C although other approved temperatures can be specified.

Extended controlled temperature conditions (ECTC): approved short-term temperature conditions, above those defined for long-term storage, transportation and use, for a given product immediately prior to administration. Any temperatures above the approved long-term storage temperatures in the cold chain could be considered for ECTC application. The development of CTC terminology and the proposing of the alternative ECTC terminology are described in the Ottawa meeting report (8).

Product-release model: a model that describes the relationship between release and expiry specifications to ensure that the product will meet defined specifications throughout its shelf-life.

Quality attributes: physical, chemical, biological, biopharmaceutical and microbiological attributes that can be defined, measured and continually monitored to ensure that final product outputs remain within acceptable quality limits.

Real-time and real-condition stability studies: studies of the physical, chemical, biological, biopharmaceutical and microbiological characteristics of a vaccine, during and up to the expected shelf-life and storage periods of samples kept under expected handling and storage conditions. The results obtained are used to recommend storage conditions and to establish the shelf-life and/or release specifications.

Shelf-life: the period of time during which a vaccine, when stored under approved conditions, is expected to comply with the specifications. The shelf-life is determined by stability studies on a number of product batches and is used to establish the expiry date of each batch of a final product.

Stability of vaccine: the ability of a vaccine to retain its physical, chemical, biological, biopharmaceutical and microbiological properties within specified limits to assure clinical performance throughout its shelf-life.

Stability-indicating parameters: quality parameters (direct or indirect indicators of vaccine efficacy or safety) that are sensitive to storage conditions. These parameters are used in stability studies to assure product quality throughout
the shelf-life. Determination of these parameters should result in quantitative values with a detectable rate of change. Qualitative parameters such as sterility may also be considered but cannot be included in the statistical analysis.

**WHO controlled temperature chain (CTC) programme:** a specific approach to vaccine management that allows vaccines to be kept at temperatures above the long-term storage condition for a limited period of time under monitored and controlled conditions appropriate to the stability of the antigen. Current WHO programme conditions for CTC include a single exposure just prior to administration, tolerating ambient temperatures of at least 40 °C for a limited duration of at least 3 days, with these temperature and time conditions included in the approved label.

**4. General considerations for the evaluation of vaccines for use under ECTC**

The use of vaccines under ECTC requires an appropriate vaccine stability assessment and consideration of the feasibility of compliance with the approved storage conditions in the field. While the stability evaluation principles described here could potentially be applied to data to support multiple temperature exposures for a vaccine, consideration would then need to be given to how such exposures would be tracked for specific vaccine final containers. When reviewing the potential difficulties in tracking multiple exposures, and ensuring that final containers that have reached the maximum exposure limit are discarded, it was concluded that at this time guidance for an ECTC label should be limited to a single planned exposure of specified duration within the labelled expiry date. Hence, once a vaccine has been stored under ECTC, it should not be returned to normal cold chain storage (for example, at 2–8 °C) in order to prevent the inadvertent administration of vaccine that is potentially out of specification. As experience with ECTC stability assessment and programme implementation expands, this conclusion could potentially be reconsidered in a future guidance update.

An ECTC application could potentially be approved solely on the basis of product-specific stability and other quality data when both of the following conditions are met:

- the approved product specifications, supported by quality attributes of the clinical lots, remain unchanged and the vaccine is expected to be compliant with these specifications following normal storage for the full shelf-life, including the ECTC exposure;
- the battery of tests performed to assess vaccine stability, which may include additional characterization assays in specific cases, has
the capacity to detect changes in potency and/or immunogenicity as well as safety parameters that are predictive of vaccine clinical performance.

Additionally, when a manufacturer has accelerated stability data that bracket the intended ECTC exposure (for example, 40 °C), the potential for interpolation of the data to estimate the decay rate at the target ECTC temperature could be considered on a case-by-case basis. It should be noted that the accelerated stability data must be from lots that represent the current manufacturing process. Vaccines used under ECTC should be capable of withstanding the approved planned exposure conditions regardless of the shelf-life remaining before expiry. These evaluations must involve statistical analysis of stability data to determine the rates of decay under both the approved long-term storage conditions and those of an ECTC exposure. It is essential that adequate potency is available to compensate for any decay over the full approved shelf-life under approved long-term storage conditions, as well as under the planned ECTC exposure (for example, 40 °C for at least 3 days). Consideration of both potency requirements is necessary to address worst-case scenarios where the planned exposure occurs within the shelf-life of a vaccine lot that was filled at or near the minimum release potency (MRP).

The focus of the current document is on the assessment of potency over the shelf-life of a vaccine given the anticipated loss of potency under ECTC. However, it is also recognized that similar principles may be used to ensure that potentially unsafe degradation products do not exceed approved limits. If exposure to ECTC leads to safety concerns such as undesired degradation products or the potential reversion of toxoids under these accelerated temperature conditions, then such risks should be explicitly evaluated.

Product-specific potency evaluations should be based on decay rates, MRP and an appropriate end-of-shelf-life lower limit (LL) supported by clinical data or experience. This is described both in existing stability guidance and subsequent papers (10, 11) and is critical for ECTC applications, to which the same principles apply. A product-release model (Fig. 1) should be developed on the basis of studies using the manufacturer's assays, along with quality data and other essential information. Labelling a vaccine for ECTC use will require the support of the manufacturer and the approval of the appropriate regulatory authorities.

Fig. 1 illustrates the relationship between the MRP specification of 50 ELISA Units (EU) and the shelf-life (24 months) given the rate of decay (slope) of the potency over both the long-term storage temperature (2–8 °C) and the maximum ECTC temperature (40 °C), and indicates that the vaccine is above the approved LL for the potency (30 EU; supported by clinical lots) at the end of shelf-life. The potency decay assessment should be based on appropriate
statistical analysis of multiple lots with a given degree of confidence (for example, 95%) and should include assay variability (not shown in the figure). As noted below in section 5, logarithmic transformation (log-transformation) of potency data typically permits analysis of stability data by linear regression.

Fig. 1
Graphic representation of a product-release model for an ECTC application

Potency assays used in the assessment of stability should be fit-for-use and are typically validated as accurate, sensitive, robust and stability-indicating. Given the greater chance of product failure after exposure to the ECTC temperatures being considered, the ability of an assay (and even of existing approved assays) to detect quality-related outcomes associated with an ECTC exposure should be re-evaluated. In some cases, supplementary potency assays or key stability-indicating tests linked with the clinical performance of the vaccine should be used. For example, use of the test to ascertain the percentage of free polysaccharide (free PS) in glycoconjugate vaccines should be considered, if not already performed. Other assays, such as those typically performed during product stability testing, should also be considered for use in an ECTC context. These may include assessments of quality attributes that may themselves affect stability (for example, moisture and pH), as well as tests of container integrity under the ECTC (for example, sterility and specific container integrity tests). It is not possible to perform decay modelling on products with potency assays that
have binary outputs (for example, pass/fail). In such cases, supplemental potency assays that are capable of showing the decay of the product’s active ingredient (or that can provide a worst-case estimate of that decay) may be considered for use in ECTC evaluation, recognizing the need for conservatism in interpreting the analysis and its results. In the absence of adequate stability-indicating assays for a vaccine, approval of an ECTC label would not be possible solely on the basis of a quality data assessment.

While ECTC approval is not a recommendation for shipping or storage, and unplanned excursions are not within the scope of this document, an approved ECTC label could potentially be taken into account when making decisions on product use in cases of temporary temperature excursions. However, given the finite nature of available potency over the shelf-life of any given vaccine, potency lost through unplanned excursions with specific final containers earlier in the shelf-life would not be available to support the use of those same containers of vaccines during a planned ECTC exposure later in the shelf-life. This highlights the importance of maintaining the cold chain prior to the extreme temperature conditions that the vaccine would be subjected to during a planned ECTC exposure.

For multivalent vaccines, ECTC evaluations must consider all antigens in the product. If one antigen is known to be less stable than other antigens within a specific vaccine then the suitability of the product for ECTC should be based on the least-stable antigen. Potential interference between vaccine components, including adjuvants, stabilizers and preservatives, may also need to be considered, as applicable. To simplify the discussion here, with the exception of comments related to free PS, the focus of this and subsequent sections will be on how to evaluate and manage the available potency of a monovalent vaccine.

At release, a product must possess sufficient potency to ensure clinical effectiveness throughout its shelf-life and to account for assay variability as well as any product decay. If there is insufficient potency available to permit ECTC use, or if it is desirable to extend the time of the ECTC storage condition, several strategies could be considered for enhancing the ECTC potential of a vaccine. For example, the shelf-life under the approved long-term storage conditions could be reduced in order to increase the available potency that could be applied to an ECTC exposure. An example of the use of shelf-life reduction to extend the ECTC storage time is shown in the Appendix below. If such an approach were used, it would be important to assign a unique product name to the ECTC version of the vaccine to avoid confusion in the field. The case study provided in the Appendix also illustrates that the implementation of a lower release specification for free PS would create a larger differential between the release and end-of-shelf-life specifications, which would enhance the ECTC potential of the product. Enhanced ECTC potential could also be achieved by reducing
assay variability, thus reducing the amount of potency required to account for potential errors in initial potency assignment. Finally, with a more accurate characterization of vaccine stability, a manufacturer could reduce the amount of potency required to account for errors in the estimation of shelf-life.

In general, additional clinical assessment for a previously approved product being considered in an ECTC application should be required only where a planned ECTC exposure results in a change of product specifications. For example, a lower end-of-shelf-life potency or a higher release specification that is not supported by clinical experience would potentially require further clinical evaluation. If additional clinical studies could demonstrate that lower potencies were still effective, or that higher-than-approved target release potencies would not result in new or more-frequent adverse events, a manufacturer could submit a regulatory amendment and use the broader potency ranges gained through these approaches to extend the ECTC potential of a product. Field studies in which the clinical evaluation of a vaccine has been performed on a product that has been exposed to temperatures higher than the approved conditions, but without potency and quality testing using the manufacturer’s assays, are not considered acceptable from a regulatory perspective. Clinical studies that are intended to support ECTC applications should be performed using a vaccine with known (or modelled) potency at the time of the ECTC use, as determined by the manufacturer’s assays.

Finally, when a lyophilized vaccine is being considered for ECTC applications, a stability analysis should be performed for the reconstituted product using the rigorous statistical evaluation principles described in this document. In situations where exposure to an ECTC storage condition could result in changes to the visual appearance of the lyophilized product that do not impact on its clinical performance, the manufacturer should provide relevant supportive data to the NRA considering the ECTC label change. If approved, a description of the potential change(s) in visual appearance should be included in the product leaflet and/or package insert. For liquid multi-dose vials, additional data would be required to demonstrate the antimicrobial effectiveness of the preservative under ECTC.

5. Stability evaluation of vaccines for use under ECTC

Stability evaluation of a specific vaccine planned for use under ECTC must generate sufficient scientifically valid data to support regulatory approval of labelling for such use. This requires assurance that there is sufficient potency available, even with lots near to expiry, to allow for an exposure under ECTC. The best prediction of actual end-expiry potency of any given lot depends on a variety of factors, including release potency of the specific lot, accuracy and precision of the potency assay, and the results of stability studies. Consequently,
statistical evaluation is needed to be able to state that, with a given (usually 95%) degree of confidence, the potency after ECTC exposure at expiry will still be above the minimum threshold needed for product efficacy. It is only through the use of statistical analysis that it is possible to obtain an indication of the level of confidence in results reported at release or in potencies delivered to the vaccine recipient. Therefore, statistical analysis is required to assure the quality of vaccines intended for delivery in the context of ECTC. Because the major ECTC-related concern is usually that the temperature exposure will reduce potency to unacceptable levels, the following guidance focuses on ensuring that the minimum required potency is maintained.

Even when products are sufficiently stable to tolerate an ECTC exposure, poorly designed studies or inappropriate statistical analyses can reduce the likelihood that an ECTC exposure is justified. This section describes study-design and statistical approaches that will improve the likelihood that ECTC exposure can be justified using sound scientific principles.

Although additional clinical studies will not be required for an ECTC approval for most licensed products, it is essential to establish the minimum potency specification for a specific vaccine, through initial licensing studies, for all stability assessments. Changes in specifications (including lowering of end-expiry potency specifications) may require supporting clinical data. Thus, the data package for ECTC applications should include summaries of the initial clinical studies, including the quality data for the clinical lots, to support the end-of-shelf-life potency specification.

The data package should also include stability studies that formally demonstrate that the minimum potency is achieved throughout the time to expiry, including the ECTC exposure. Estimates of the rate (or slope) at which the potency decays (hereafter, referred to as “stability estimates”) at the normal and ECTC temperatures – and an understanding of potential errors in those estimates – are the most important outcomes of the stability studies. The reliability of these stability estimates, and the extent to which the release potency of any lot can be reliably determined, depend in turn on the potency assay.

As mentioned above in section 4, stability studies to support vaccine use in an ECTC context should use the manufacturer’s potency assay in order to preserve a connection between the released product proposed for ECTC use and the original clinical material used to support product efficacy. It is likely that key parameters of the potency assay will already be known from assay validation, including assay accuracy and precision. More reliable estimates of in-use assay precision may sometimes be obtained by other means (for example, by comparing actual with modelled results in the stability analyses). Other data may also be relevant to the estimation of in-use assay precision. Because there are several possible estimates of assay precision that could be used for ECTC-related calculations, the choice of estimate should be scientifically justified; if
a clear justification cannot be made, the more conservative estimate (from the perspective of the ECTC label) should be used.

Statistical analysis of vaccine stability is normally based on a mathematical model and supported by data that describe the kinetics of potency changes at different temperatures for different periods of time. Statistical release models must support the conclusion that the mean potency of final containers in a given lot will, given all stability losses, meet specifications throughout the shelf-life with a given level of confidence (usually 95%), including permitted storage periods outside the long-term storage conditions. Typically, the rate of change (generally loss) of vaccine potency is not a simple linear function of time. Log-transformation of potency data usually leads to a more predictive model that permits analysis of stability data by linear regression. Thus, in most cases, potency data should be log-transformed before analysis. When log-transformation is not used, scientific justification for the use of a more relevant model should be provided. Log-transformation usually provides a preferred model of the biological process (since for most substances the rate of decay at any point in time depends on the quantity of substance present at that time) as compared with direct analysis of non-log-transformed potency data.

Moreover, empirical observation supports the conclusion that potency decay for many vaccines follows first-order kinetics, which are linear following log-transformation – though low precision of the potency assay may make it more difficult to determine whether stability results follow the decay model. In addition, potency measurements are often log-normally distributed; when this is the case, log-transformation may be required to satisfy the statistical assumptions of the modelling, and can further improve the precision of the stability estimate. In all cases, the decay model used should correspond to actual product decay kinetics as observed in stability studies, and this may support the use of non-log-transformed decay models (including linear models) if these models can also be justified as biologically relevant. It should be noted that log-transformation is not always the best approach for stability-indicating assays. For example, increases in degradation products over time usually cannot be modelled on a log scale. Visual examination of the plot of transformed and untransformed stability data can provide an indication of whether mathematical transformations can linearize the decay curve. Sometimes no biologically meaningful model can be identified that fits the data, as may occur when there are multiple phases in the decay kinetics. In this case, decay estimates during ECTC exposures can be estimated by using only the beginning and end results of the stability testing. If the most appropriate choice of model is unclear, selection of the most conservative option is appropriate.

Stability studies should properly evaluate the kinetics of decay, and should indicate that decay rates (after any transformation) are not higher at the end of the observation period than at the beginning. These studies should
include a sufficient number of time points to determine the adequacy of the decay model, while also providing robust stability estimates. Linearity can theoretically be supported by using at least three time points – the starting point, the end-point (corresponding to the desired ECTC exposure time) and ideally the midpoint. However, when assay precision is not high, additional time points will probably be needed to increase the degree of assurance that the change in ECTC-related stability parameters truly follows a linear model. Inclusion of additional time points can also improve estimates of the true rate of change in potency. If linearity has already been established, more precise estimates of decay rates under ECTC can be obtained by testing sufficient numbers of samples at post-ECTC exposure time points as compared to pre-ECTC exposure time points. When decay kinetics are linear, testing at time points beyond the proposed ECTC use can also improve the precision of the stability estimate. It is often assumed that decay rates under ECTC will be similar near the time of release and at expiry, but (as with the rate of decay over the normal storage period) this assumption should be verified. If the rate of decay does vary depending on the time from release then modelling may need to take this into account, along with consideration of the potential uncertainties added by any assumptions that are made. Testing larger numbers of independent samples (batches/lots) can further improve this precision and can potentially increase the likelihood that these studies will support ECTC use, but in all cases a minimum of three lots should be tested.

Typical stability evaluations often include an analysis for “poolability”. The presence of one or more outliers in a stability-indicating assay may indicate unacceptable manufacturing variability and/or could cause a combined decay slope calculated using a small number of lots to be inaccurate. Previous guidance advocated using the worst-case lot for the decay slope estimate when analysis suggested that data from tested lots may not be poolable. However, using the worst-case lot can inappropriately penalize expected variability (over which the manufacturer has no control) and can be a disincentive to conducting more complete testing. It is reasonable to include all tested lots in the stability analysis so long as these lots are considered representative of the licensed (and “in-control”) manufacturing process, and so long as a sufficient number of lots are included to address random variability. Pooling of data from a sufficient number of representative lots should be statistically justified and agreed with the NRA.

Stability testing normally provides information on expected rates of decay (for the linear model, the decay slope) and standard error of the decay slope at “n” different temperatures of exposure (modelling storage, shipping, post-reconstitution and so on) as well as under ECTC (for time $t_{ECTC}$ with decay slope $b_{ECTC}$). Modelling of stability test results can also provide an estimate of the precision of the potency assay.
A first approximation of the impact of an ECTC exposure is the 95% confidence bound on the expected loss in potency $L_{ECTC}$ as a result of the ECTC exposure. This can be estimated as:

$$L_{ECTC} = -t_{ECTC} \cdot b_{ECTC} + z_{1-\alpha} \cdot (t_{ECTC} \cdot s(b_{ECTC}))$$

...where $t_{ECTC}$ is the time at ECTC temperatures; $b_{ECTC}$ is the decay slope (a negative number) at ECTC temperatures; $z_{1-\alpha}$ is the one-sided $z$ statistic at the confidence level associated with the desired degree of confidence ($\alpha = 0.05$ for 95% confidence bounds); and $s(b_{ECTC})$ is the standard error of the decay slope. If this amount of additional decay in potency beyond that considered by the product-release model is considered acceptable, the product can be accepted for ECTC use.

A more accurate and less conservative estimate can be obtained by calculating the aggregate error associated with all assumptions in the decay model. The product-release model shown above in Fig. 1 defines the needed potency of the product at the time of release. From this information, it is possible to calculate the statistical lower bound (LB) on the mean potency of a product that is released at the minimum release potency and that is exposed to multiple temperature conditions. This is shown as follows:

$$LB_{1-\alpha} = MRP + t_1 \cdot b_1 + t_2 \cdot b_2 + \ldots + t_n \cdot b_n + t_{ECTC} \cdot b_{ECTC} - U$$

...where $1-\alpha$ describes the statistical confidence level associated with the lower bound ($\alpha = 0.05$ for 95% confidence bounds); $MRP$ is the manufacturer’s minimum allowable release potency (usually log-transformed); $t_i$ is time at temperature $i$ (where $i$ is a positive integer used to represent the series of temperatures to which the vaccine may be exposed); $b_i$ is decay slope (a negative number, or zero if positive) at temperature $i$; and $U$ is the combined uncertainty associated with the independent estimation of the numbers on the right side of the equation. Typically:

$$U = z_{1-\alpha} \cdot \sqrt{(s_{assay})^2 + (t_1 \cdot s(b_1))^2 + (t_2 \cdot s(b_2))^2 + \ldots + (t_n \cdot s(b_n))^2 + (t_{ECTC} \cdot s(b_{ECTC}))^2}$$

...where $z_{1-\alpha}$ is the one sided $z$ statistic at the confidence level associated with the desired degree of confidence ($\alpha = 0.05$ for 95% confidence bounds); $s_{assay}$ is the assay precision; and $s(b_i)$ is the precision (standard error) of the

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1 As noted, the equations listed are for the general case that could include modelling storage, shipping, post-reconstitution and so on. However, when only considering the normal storage condition and a single ECTC exposure, an example of a simplified form of the equations would be: $LB_{1-\alpha} = MRP + t_1 \cdot b_1 + t_{ECTC} \cdot b_{ECTC} - U$. 


decay slope at temperature \( i \). Thus, the expected end-expiry potency is expected to be (with 95% confidence) as low as \( \text{LB}_{0.95} \) – which accounts for the manufacturer’s MRP, the estimated potency losses at the various temperatures and the associated uncertainty.

Without an ECTC exposure, and with omission of the ECTC-associated terms, the above equations yield the minimum potency that an already-licensed product is expected to maintain based on the manufacturer’s release model, throughout its time to expiry. Inclusion of the ECTC term allows a reviewer to determine the degree to which potency is affected by the ECTC exposure and whether or not that is acceptable on the basis of clinical experience with the vaccine at that level of potency.

When the LL of potency, defined as the minimum potency below which there is concern about product efficacy (considering the potency results from the clinical lots and post-market experience) has been defined, it is preferable to rearrange the terms of the above equation to determine the MRP required to maintain potency through to expiry, including the ECTC exposure. In essence, this means calculating the minimum amount of potency that must be added to that minimum potency (LL) in order to assure product quality throughout normal storage and handling, including ECTC exposure, as follows:

\[
\text{MRP} = \text{LL} - t_1 \cdot b_1 - t_2 \cdot b_2 - \ldots - t_n \cdot b_n - t_{\text{ECTC}} \cdot b_{\text{ECTC}} + U
\]

The product may be released at a higher potency within the approved specification which provides a convenient way to ensure that the release model will support ECTC labelling. If ECTC exposure potential cannot be established then several options could be considered, as outlined above in section 4 (see also the definition of Product-release model in section 3 and the related Fig. 1 in section 4). It should be noted that the analytical principles represented in the equations above are the same as those in existing WHO vaccine stability guidance (1) and that they have been expanded to include ECTC exposure. It should also be noted that the equations here are not the only ways to represent these calculations and that other approaches that encompass similar statistical principles could potentially be acceptable where justified.

6. Monitoring ECTC

All vaccines should be kept under the recommended long-term storage conditions with appropriate oversight prior to ECTC exposure. Use of vaccines under ECTC requires specific monitoring of temperature exposure (for example, peak threshold indicator) and time, as well as formal procedures to ensure that the approved maximum temperature and time are not exceeded. Unused vaccines that exceed the approved maximum temperature or time should be
disposed of by suitable procedures. ECTC temperature-monitoring systems need to be able to distinguish vaccines that are still appropriate for use from vaccines that have exceeded the limits imposed by the data supporting ECTC use. The monitoring requirements for ECTC differ from those for long-term storage and transport. The respective monitoring systems for long-term and ECTC storage should be consistent with product stability characteristics. In order to allow an ECTC exposure, the monitoring system should assure that approved long-term storage conditions, especially with respect to temperature, are not exceeded. Prior to vaccine approval for use under ECTC, the relevant stakeholders, which may include the manufacturer, the NRA and the immunization programme, should work together to ensure that an appropriate monitoring system is in place.

7. Suggested product labelling information for use under ECTC

ECTC should be described in the product leaflet and/or package insert in order to provide information to medical practitioners. The statement on ECTC should appear in a separate paragraph in the appropriate section of the label (for example, Storage and Handling).

The ECTC information in the product leaflet and/or package insert should be clear, concise and specific. If a vaccine consists of two or more components (for example, lyophilized vaccine and diluent) then ECTC information should be given for all of the components of the vaccine.

Information to be included in an ECTC statement should take account of the following, if applicable:

- maximum allowed temperature
- maximum time allowed at a specific temperature
- in-use shelf-life after opening (or reconstitution or mixture, if applicable)
- advice on unopened vials exposed to ECTC (for example, on disposal)
- that only a single ECTC exposure directly prior to use or disposal, and within the shelf-life, is permitted.

The following may serve as a model text for the product leaflet and/or package insert text for ECTC use: *The vaccine [and its diluent/solvent or other component] may be kept for a single period of time of up to [x days or x weeks or x months] at temperatures of up to [x °C] immediately prior to administration. At the end of this period, the vaccine [must be disposed of]. This is not a recommendation*
for storage but is intended to guide decision-making when exposure to higher temperatures is planned. [After opening [or reconstitution or mixture], the vaccine can be kept for [x hours or x days] at temperatures of up to [x °C] at which point it must be disposed of].

8. Authors and acknowledgments

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Canada; Dr K. Brusselmans, Scientific Institute of Public Health, Belgium; Dr M. Chultem, Health Canada, Canada; Dr C. Cichutek, Paul-Ehrlich-Institut, Germany; Mr W. Conklin, Merck, the USA; Dr C. Conrad, Paul-Ehrlich-Institut, Germany; Ms D. Doucet, GlaxoSmithKline Biologicals SA, Belgium; Dr W. Egan, Novartis, the USA; Dr L. Elmgren, Health Canada, Canada; Dr D. Felnerova, Crucell Switzerland AG, Switzerland; Dr S. Gairola, Serum Institute of India Ltd, India; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Dr D.A. Hokama, BioManguinhos, Brazil; Dr W. Huang, Xiamen Innovax Biotech Co., China; Mrs T. Jivapaisarnpong, Department of Medical Sciences, Thailand; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr P. Krause, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr H. Langar, WHO Regional Office for the Eastern Mediterranean, Egypt; Dr A. Laschi, Sanofi Pasteur, France; Dr C. Lecomte, GlaxoSmithKline Vaccines, Belgium; Professor H. Leng, Consultant, Somerset West, South Africa; Dr M. (Ferguson) Lennon, Consultant, Horning, the United Kingdom; Ms A. Lopez, Biológicos y Reactivos de México S.A. de C.V., Mexico; Dr A. Luethi, Crucell Switzerland AG, Switzerland; Dr W. Matheis, Paul-Ehrlich-Institut, Germany; Dr A. Merkle, Paul-Ehrlich-Institut, Germany; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr B.L.M. Moreira, Agência Nacional de Vigilância Sanitária, Brazil; Dr K-T. Nam, Ministry of Food and Drug Safety, Republic of Korea; Dr R. Nibbeling, Institute for Translational Vaccinology, Netherlands; Dr V. Oeppling, Paul-Ehrlich-Institut, Germany; Dr D.M. Pascual, Centro para el Control Estatal de la Calidad de los Medicamentos, Cuba; Dr M. Pfleiderer, Paul-Ehrlich-Institut, Germany; Dr M.L. Pombo, Pan American Health Organization, the USA; Dr T. Prusik, Temptime Corporation, the USA; Dr M. Reers, Biological E Ltd, India; Mr T. Schofield, MedImmune, the USA; Ms F.A. Setyorini, PT Bio Farma, Indonesia; Dr S. Shani, Food and Drug Administration, India; Dr I.S. Shin, Ministry of Food and Drug Safety, Republic of Korea; Dr S.C. Da Silveira, Agência Nacional de Vigilância Sanitária, Brazil; Dr D. Smith, Health Canada, Canada; Dr M. Vega, Centre for Genetic Engineering and Biotechnology, Cuba; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; Ms S. Wong, Merck, the USA; Dr T. Wu, Health Canada, Canada; Dr M. Zeng, National Institutes for Food and Drug Control, China; and Dr J. Kim, Dr D.G. Maire, Dr C.A. Rodriguez-Hernandez, Dr J. Shin and Ms S. Zipursky, World Health Organization, Switzerland.

The second draft document (WHO/BS/2015.2268) was prepared by Dr C. Conrad, Paul-Ehrlich-Institut, Germany; Dr I. Feavers, National Institute for Biological Standards and Control, the United Kingdom; Dr K. Gao, World Health Organization, Switzerland; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Mrs T. Jivapaisarnpong, Department of Medical
Sciences, Thailand; Dr J. Kim, World Health Organization, Switzerland; Dr I. Knezevic, World Health Organization, Switzerland; Dr P. Krause, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr J. Shin, WHO Regional Office for the Western Pacific, Philippines; Dr D. Smith, Health Canada, Canada; Dr J. Southern, Adviser to the Medicines Control Council of South Africa, South Africa; Mr M. Walsh, Health Canada, Canada; Dr T. Wu, Health Canada, Canada, taking into account comments received from: Dr B.D. Akanmori, WHO Regional Office for Africa, Congo; Dr A. Alsalhani, Médecins Sans Frontières, France; Dr M-C. Annequin, Agence nationale de sécurité du médicament et des produits de santé, France; Dr B. Bolgiano, National Institute for Biological Standards and Control, the United Kingdom; Dr J. Bridgewater, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr A. Chang, Johns Hopkins University, the USA; Dr A. Cheung, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Ms D. Doucet, GlaxoSmithKline Biologicals SA, Belgium; Dr J. Du, National Institutes for Food and Drug Control, China; Dr W. Egan, Novartis, the USA; Dr S. Gagneten, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr D. Garcia, Agence nationale de sécurité du médicament et des produits de santé, France; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Dr T. Guo, National Institutes for Food and Drug Control, China; Mr K. Hicks (International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) representative), Sanofi Pasteur, France; Ms A. Juan-Giner, Médecins Sans Frontières, France; Dr B-G. Kim, Ministry of Food and Drug Safety, Republic of Korea; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr T-L. Lin, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr M. Ramos, Public Health England, the United Kingdom; Mr T. Schofield, MedImmune, the USA; Dr J. Shin, WHO Regional Office for the Western Pacific, Philippines; Dr S.C. Da Silveira Andreoli, Agência Nacional de Vigilância Sanitária, Brazil; Dr D. Smith, Health Canada, Canada; Dr T. Prusik, Temptime Corporation, the USA; and Ms A-L. Kahn, Dr U. Kartoglu, Dr D. Petit and Ms S. Zipursky, World Health Organization, Switzerland.

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for Biological Standards and Control, the United Kingdom; Dr S. Gairola, Serum Institute of India, India; Mr K. Gopinathan, Biological E Ltd, India; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Dr K. Hicks, Sanofi Pasteur, France; Mr Y. Kaushik, Bharat Biotech International, India; Dr B-G. Kim, Ministry of Food and Drug Safety, Republic of Korea; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr P. Krause, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Mr A. Kukrety, Food and Drug Administration, India; Dr J.S. Lloyd, Immunization Supply Chain, France; Dr A. Luethi, Crucell Switzerland AG, Switzerland; Dr B.L.M. Moreira, Agência Nacional de Vigilância Sanitária, Brazil; Dr D.M. Pascual, Centro para el Control Estatal de la Calidad de los Medicamentos, Cuba; Mr C. Perrin, Médecins Sans Frontières, France; Dr T. Prusik, Temptime Corporation, the USA; Mrs J. Rogers, Food and Drugs Authority, Ghana; Ms P.P. Said, PT Bio Farma, Indonesia; Ms C. Schmit, GlaxoSmithKline Biologics SA, Belgium; Mr T. Schofield, MedImmune, the USA; Dr D. Smith, Health Canada, Canada; Dr J. Southern, Adviser to the Medicines Control Council of South Africa, South Africa; Ms S. Wong, Merck, the USA; Dr T. Wu, Health Canada, Canada; and Ms A-L. Kahn, Dr U. Kartoglu, Dr J. Kim, Dr I. Knezevic, Dr A. Meek and Dr C.A. Rodriguez-Hernandez, World Health Organization, Switzerland.

Further changes were subsequently made to document WHO/BS/2015.2268 by the WHO Expert Committee on Biological Standardization.

9. References


Appendix

Product-specific ECTC evaluation of a model monovalent polysaccharide conjugate vaccine

The model vaccine and the stability data presented in this appendix were developed on the basis of Health Canada's overall experience with conjugate vaccines and do not represent characteristics or data from any specific product. The analysis presented is also applicable to other stability-indicating parameters, such as vaccine potency. The vaccine example under evaluation is a monovalent conjugate vaccine composed of purified capsular polysaccharide (PS) covalently attached to diphtheria toxoid protein. The final vaccine product is a non-adjuvanted liquid formulation presented in single-dose vials. The normal storage temperature for this model conjugate vaccine is 2–8 °C, with a time to expiry of three years; the temperature under consideration for the ECTC application is 40 °C. The quality attributes monitored in routine stability studies intended for licensure included total PS, free PS, molecular size distribution, free protein, pH and sterility. Free PS is considered a key stability-indicating attribute for polysaccharide conjugate vaccines since in general this parameter is linked to the clinical performance of this type of vaccine. The specification for free PS for this model conjugate vaccine was set as “not more than (NMT) 15%” at release and “NMT 25%” at the end of the shelf-life. A review of manufacturing data indicated that, at release, 90% of the commercial lots contained less than 10% of free PS and 10% of lots contained free PS in the range 10–13%. In addition, vaccine lots containing 5–25% free PS were shown to be safe and immunogenic in clinical studies.

Stability data

Real-time and real-condition stability studies were conducted to establish the shelf-life under normal storage conditions (2–8 °C) and to support the ECTC application. Although a minimum of three lots is required for statistical modelling, analysis of a larger data set (more lots) leads to more-precise estimates. In this example, routine stability-monitoring tests were performed for four commercial vaccine lots stored at 2–8 °C and for an additional four commercial lots stored at 40 °C. In addition, O-acetyl content, nuclear magnetic resonance (NMR) spectrum and immunogenicity (rabbit complement source serum bactericidal assay and immunoglobulin G) in a mouse model were also evaluated to characterize vaccine lots exposed to the 40 °C condition. Analysis
of routine monitoring data revealed that total PS, molecular size distribution, free protein and pH were stable for all lots stored under the 2–8 °C and 40 °C conditions. However, an increase of free PS was observed for all lots, as summarized in Tables 1 and 2.

Table 1
Summary of free PS content at 2–8 °C

<table>
<thead>
<tr>
<th>Lot #</th>
<th>Free PS (NMT 25%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>7.53</td>
</tr>
<tr>
<td>2</td>
<td>7.01</td>
</tr>
<tr>
<td>3</td>
<td>2.38</td>
</tr>
<tr>
<td>4</td>
<td>5.71</td>
</tr>
</tbody>
</table>

NMT = not more than; M = month; NT = not tested.

Table 2
Summary of free PS content at 40 °C

<table>
<thead>
<tr>
<th>Lot #</th>
<th>Free PS (NMT 25%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1.74</td>
</tr>
<tr>
<td>7</td>
<td>5.43</td>
</tr>
<tr>
<td>8</td>
<td>5.21</td>
</tr>
</tbody>
</table>

NMT = not more than; W = week.

Statistical analysis

An initial analysis (1) demonstrated that the free PS data did not fit a linear regression, with or without log-transformation (plots not provided). Because the increase in the stability-indicating free PS is due to the hydrolysis of bound PS, the rate of increase of free PS is the same as the rate of decrease of bound PS. Therefore, the bound PS at each test point can be calculated from the free and total PS on the basis of mass balance. The hydrolysis of bound PS can be analysed as a first-order reaction at a decay rate that is proportional to the concentration
of bound PS. Consequently, the rate of increase of free PS was analysed indirectly through the modelling of bound PS, and log-transformation of the bound PS content at different test points yielded data that were more amenable to linear regression analysis (1). Thus, free PS data obtained in stability studies were converted to percentage bound PS (Tables 3 and 4) and then subjected to log-transformation. A release model was developed to characterize the relationship between bound PS at release and end-expiry, thus permitting evaluation of potential ECTC use.

Table 3
Summary of bound PS content at 2–8 °C

<table>
<thead>
<tr>
<th>Lot #</th>
<th>Bound PS (NLT 75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>92.47</td>
</tr>
<tr>
<td>2</td>
<td>92.99</td>
</tr>
<tr>
<td>3</td>
<td>97.62</td>
</tr>
<tr>
<td>4</td>
<td>94.29</td>
</tr>
</tbody>
</table>

NLT = not less than; M = month; NT = not tested.

Table 4
Summary of bound PS content at 40 °C

<table>
<thead>
<tr>
<th>Lot #</th>
<th>Bound PS (NLT 75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>97.99</td>
</tr>
<tr>
<td>6</td>
<td>98.26</td>
</tr>
<tr>
<td>7</td>
<td>94.57</td>
</tr>
<tr>
<td>8</td>
<td>94.79</td>
</tr>
</tbody>
</table>

NLT = not less than; W = week.

Statistical analysis was performed using R version 3.1.1 (2) to estimate the loss of bound PS under both the 2–8 °C and 40 °C storage conditions with 95% confidence, and the key results are summarized in Tables 5 and 6. The analysis was undertaken in the following steps:
1. For each stability lot, the percentage of bound PS at each test point was log-transformed and the slope was calculated using a linear regression model. Plots of the linear regression fit for all stability lots are presented in Fig. A1.

2. Lots 1, 2, 3 and 4, monitored at 2–8 °C, were assessed with respect to slope variability, which was considered acceptable for use of the linear regression model with a pooled (mean) slope for all four lots. The same analysis was also applied to the data set (lots 5, 6, 7 and 8) at 40 °C, which supported the use of a pooled slope.

3. The assay precision ($s_{assay}$) was estimated by the residual error from the regression analysis using the pooled slope for the corresponding data set.

4. The uncertainty was calculated using formulae described above in section 5. Two examples are:

   - the uncertainty ($U$) at 2–8 °C for 36 months = $z_{0.95} \cdot \sqrt{(s_{assay})^2 + (t_{2-8} \cdot s(b_{2-8}))^2}$
     $= 1.644854 \cdot \sqrt{[0.012728112 + (36 \cdot 0.0001845235)^2]} = 0.02361$.

   - $U$ at 2–8 °C for 36 months followed by 3 days at 40 °C
     $= z_{0.95} \cdot \sqrt{(s_{assay})^2 + (t_{2-8} \cdot s(b_{2-8}))^2 + (t_{ECTC} \cdot s(b_{ECTC}))^2}$
     $= 1.644854 \cdot \sqrt{[0.012728112 + (36 \cdot 0.0001845235)^2 + (0.1 \cdot 0.008625)^2]}$
     $= 0.02366$.

5. The change in bound PS was estimated using the linear regression model and the formula provided above in section 5. An example is provided below to illustrate the calculation of the total change in bound PS at 2–8 °C over 36 months, plus 3 days at 40 °C. This is based on the worst-case lots which contain 85% bound PS at release.

   - First step: the log-transformed total decay of bound PS:
     $= (t_{2-8} \cdot b_{2-8}) + (t_{ECTC} \cdot b_{ECTC}) - U$
     $= 36 \cdot (-0.002430492) + 0.1 \cdot (-0.07429892) - 0.02365824 = -0.1185858$.

   - Second step: the log-transformed bound PS at end of storage (2–8 °C plus ECTC):
     $= \log_e (\text{bound PS at release}) + [(t_{2-8} \cdot b_{2-8}) + (t_{ECTC} \cdot b_{ECTC}) - U]$
     $= \log_e 85 - 0.1185858 = 4.324065$.

   - Third step: bound PS at the end of storage (2–8 °C plus ECTC) = $e^{4.324065} = 75.4949$. 
- Fourth step: the decay of bound PS at 2–8 °C over 36 months plus 3 days at 40 °C:
  \[ \text{bound PS at release} - \text{bound PS at the end of storage (2–8 °C plus ECTC)} \]
  \[ = 85 - 75.4949 = 9.5051. \]

### Table 5
**Summary of statistical analysis of bound PS data at 2–8 °C**

<table>
<thead>
<tr>
<th>Data set</th>
<th>Shelf-life (months)</th>
<th>Pooled slope (per month)</th>
<th>( s(b_{2-8}) ) ( ^a )</th>
<th>( s_{\text{assay}} ) ( ^b )</th>
<th>( U ) ( ^c )</th>
<th>Decay of bound PS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 lots (0–24M)</td>
<td>24</td>
<td>-0.003311</td>
<td>0.0002765</td>
<td>0.01146</td>
<td>0.02178</td>
<td>8.1851</td>
</tr>
<tr>
<td>4 lots (0–36M)</td>
<td>36</td>
<td>-0.002430</td>
<td>0.0001845</td>
<td>0.01273</td>
<td>0.02361</td>
<td>8.9388</td>
</tr>
</tbody>
</table>

- standard error of slope.
- assay variability, estimated as standard deviation of residuals.
- combined uncertainty, calculated using the formula described above in section 5.

### Table 6
**Summary of statistical analysis of bound PS data at 2–8 °C and 40 °C**

<table>
<thead>
<tr>
<th>Data set</th>
<th>Pooled slope (per month)</th>
<th>( s(b_{40}) ) ( ^a )</th>
<th>Months at 2–8 °C</th>
<th>Days at 40 °C</th>
<th>( s_{\text{assay}} ) ( ^b )</th>
<th>( U ) ( ^c )</th>
<th>Decay of bound PS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 lots (0–12W)</td>
<td>-0.04482</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>4 lots (0–4W)</td>
<td>-0.07430</td>
<td>0.007766</td>
<td>24</td>
<td>7</td>
<td>0.01146</td>
<td>0.02199</td>
<td>9.5207</td>
</tr>
<tr>
<td></td>
<td>-0.07430</td>
<td>0.008625</td>
<td>36</td>
<td>3</td>
<td>0.01273</td>
<td>0.02366</td>
<td>9.5051</td>
</tr>
</tbody>
</table>

- standard error of slope.
- assay variability, estimated from the regression analysis residual error.
- combined uncertainty, calculated using the formula described above in section 5.
- \( W = \) week.
Examination of the plots presented in Fig. A1 indicates that at each of these temperature conditions the decay of bound PS for each lot appears to be better modelled by a straight line when compared to the modelling of free PS. This supports the use of a linear regression model of bound PS in this case. However, it is also noted that the estimated slope of bound PS decay differs over different study periods, especially under 40 °C. As shown in Table 6 the decay rate is higher over 4 weeks (−0.07430/week) compared to that over 12 weeks (−0.04482/month). Differences in rates of change for key quality attributes over a product’s shelf-life are not uncommon; therefore it is important to highlight the need to characterize trends when modelling the data and the need to estimate the rate of change based on real-time data over the full study period.

Owing to limited data points at 40 °C, the rate of bound PS decay used for ECTC application was based on the modelling of a 4-week data set, and a conservative approach was taken to limit the total decay of bound PS to slightly below 10%. The statistical analysis summarized in Table 6 revealed an estimated 9.5% loss of bound PS (equal to the increase of free PS) after 36 months storage at 2–8 °C followed by 3 days at 40 °C, or 24 months storage at 2–8 °C followed by 7 days at 40 °C.
Conclusion

The application of the “product release model” to the analysis of free PS can be summarized as:

\[
\text{Release specification (15\%) = End of shelf-life specification (25\%) minus estimated combined increase at 2–8 °C and 40 °C (upper bound with 95\% confidence level)}
\]

On the basis of statistical modelling and product-related information, the following can be concluded:

- Different specifications for release and end of shelf-life should be established for free PS for this model conjugate vaccine. A specification of “NMT 25%” at the end of shelf-life is considered acceptable on the basis of clinical lots shown to be safe and immunogenic in clinical studies. A release specification of “NMT 15%” was considered appropriate on the basis of manufacturing capability, which ensures a high compliance rate for commercial lots at release.

- A 36-month shelf-life at 2–8 °C was determined to be appropriate for this model conjugate vaccine. This conclusion ensures that worst-case lots, which contain the highest level of free PS permitted by the release specification (NMT 15%), plus the accumulation of free PS during the storage period (approximately 8.94%), comply with the end of the shelf-life specification (NMT 25%).

- A single storage period of 3 days at 40 °C, prior to immunization, was considered acceptable because the worst-case lots, which contain 15% free PS at release and are stored for almost 36 months at 2–8 °C followed by an exposure of 3 days at 40 °C, were expected to contain approximately 24.51% free PS.

- If a period longer than 3 days at 40 °C is needed, shortening the shelf-life at 2–8 °C from 36 months to 24 months would allow for a single storage period of 7 days at 40 °C. Alternatively, the release specification of “NMT 15% free PS” might be tightened (for example to “NMT 13% free PS”) on the basis of additional manufacturing experience to allow for longer than 3 days of ECTC exposure while maintaining a 3-year shelf-life at 2–8 °C.

As explained above in section 4, clinical testing of a vaccine stored under ECTC would not be necessary as long as a battery of stability-monitoring tests provided sufficient assurance that the critical quality attributes of the vaccine (such as potency) met the specifications supported by clinical experience.
Other than routine stability-monitoring assays, the following characterization tests were also performed to assess quality attributes related to vaccine clinical performance after this model conjugate vaccine was stored at 40 °C for 12 weeks: (a) O-acetyl content remained stable; (b) PS structure was confirmed using NMR; and (c) carrier protein integrity was confirmed by the results of an in vivo immunogenicity test. It was noted that the antigen dose used to immunize the mice was within the dose–response curve, indicating that the in vivo test was of acceptable sensitivity. In conclusion, the available stability data sets assessing critical quality attributes were considered sufficient to support the application of a single storage period of 3-day ECTC (40 °C) for this model conjugate vaccine, within the approved 3-year shelf-life at 2–8 °C.

References

Annex 6

Biological substances: WHO International Standards, Reference Reagents and Reference Panels


At its meeting in October 2015, the WHO Expert Committee on Biological Standardization made the changes shown below to the previous list.

### Additions

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Activity</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibiotics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin*</td>
<td>76 000 IU/vial</td>
<td>Fourth WHO International Standard</td>
</tr>
<tr>
<td><strong>Biotherapeutics other than blood products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour necrosis factor receptor Fc fusion protein (etanercept)</td>
<td>10 000 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Antibodies to erythropoietin (human)</td>
<td>Nine panel members; no unitage assigned</td>
<td>First WHO Reference Panel</td>
</tr>
<tr>
<td><strong>Blood products and related substances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood coagulation factor IX (concentrate)</td>
<td>10.5 IU/ampoule</td>
<td>Fifth WHO International Standard</td>
</tr>
</tbody>
</table>

---

1 Unless otherwise indicated, all materials are held and distributed by the National Institute for Biological Standards and Control, Potters Bar, Herts, EN6 3QG, the United Kingdom. Antibiotic reference preparations identified by an * in the above list are held and distributed by the European Directorate for the Quality of Medicines & HealthCare, Council of Europe, 7 allée Kastner, CS 30026 F-67081, Strasbourg, France. Materials identified by an ** in the above list are held and distributed by the Paul-Ehrlich-Institut, 63225 Langen, Germany.
## In vitro diagnostic device reagents

<table>
<thead>
<tr>
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<th>Activity</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC virus DNA for NAT-based assays</td>
<td>7.0 log_{10} IU/ml</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>BK virus DNA for NAT-based assays</td>
<td>7.2 log_{10} IU/ml</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Hepatitis C virus RNA for NAT-based assays</td>
<td>5.0 log_{10} IU/vial</td>
<td>Fifth WHO International Standard</td>
</tr>
<tr>
<td>Anti-<em>Toxoplasma gondii</em> (human)</td>
<td>160 IU/ampoule (IgG content 263 U/ampoule)</td>
<td>Fourth WHO International Standard</td>
</tr>
<tr>
<td>Assignment of a holotranscobalamin value to the current First WHO International Standard for vitamin B12 and folate in human serum</td>
<td>107 pmol/l</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Human C-peptide</td>
<td>8.64 µg/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Hepatitis E virus genotypes for NAT-based assays**</td>
<td>Eleven panel members; no unitage assigned</td>
<td>First WHO Reference Panel</td>
</tr>
<tr>
<td>Extension of the First WHO Repository of platelet transfusion relevant bacterial strains**</td>
<td>Extended by 10 bacterial strains</td>
<td>First WHO Repository</td>
</tr>
<tr>
<td>Ebola virus antibodies</td>
<td>1 U/ml</td>
<td>First WHO reference reagents</td>
</tr>
<tr>
<td>Ebola virus RNA for NAT-based assays</td>
<td>7.5 log_{10} U/ml (for assays targeting the <em>np</em>, <em>vp35</em> and <em>gp</em> genes); and 7.7 log_{10} U/ml (for assays targeting the <em>vp40</em> and <em>l</em> genes)</td>
<td>First WHO reference reagents</td>
</tr>
<tr>
<td>Preparation</td>
<td>Activity</td>
<td>Status</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>---------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><strong>Vaccines and related substances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-EV71 serum (human)</td>
<td>1000 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>EV71 neutralization assay standard</td>
<td>300 IU/ampoule</td>
<td>First WHO Reference Reagent</td>
</tr>
<tr>
<td>Meningococcal serogroups A and X polysaccharide</td>
<td>0.845 ± 0.043 mg/ampoule</td>
<td>First WHO international standards</td>
</tr>
<tr>
<td></td>
<td>(for serogroup A); and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.776 ± 0.089 mg/ampoule</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(for serogroup X)</td>
<td></td>
</tr>
<tr>
<td>Diphtheria toxoid for use in flocculation test</td>
<td>1870 Lf/ampoule</td>
<td>Third WHO International Standard</td>
</tr>
</tbody>
</table>
The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO’s constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

The Organization seeks through its publications to support national health strategies and address the most pressing public health concerns of populations around the world. To respond to the needs of Member States at all levels of development, WHO publishes practical manuals, handbooks and training material for specific categories of health workers; internationally applicable guidelines and standards; reviews and analyses of health policies, programmes and research; and state-of-the-art consensus reports that offer technical advice and recommendations for decision-makers. These books are closely tied to the Organization’s priority activities, encompassing disease prevention and control, the development of equitable health systems based on primary health care, and health promotion for individuals and communities. Progress towards better health for all also demands the global dissemination and exchange of information that draws on the knowledge and experience of all WHO’s Member countries and the collaboration of world leaders in public health and the biomedical sciences.

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SELECTED WHO PUBLICATIONS OF RELATED INTEREST

WHO Expert Committee on Biological Standardization
Sixty-fifth report.
WHO Technical Report Series, No. 993, 2015 (xvi + 262 pages)

WHO Expert Committee on Biological Standardization
Sixty-fourth report.
WHO Technical Report Series, No. 987, 2014 (xviii + 266 pages)

WHO Expert Committee on Biological Standardization
Sixty-third report.
WHO Technical Report Series, No. 980, 2014 (xv + 489 pages)

WHO Expert Committee on Biological Standardization
Sixty-second report.

WHO Expert Committee on Biological Standardization
Sixtieth report.
WHO Technical Report Series, No. 977, 2013 (viii + 231 pages)

WHO Expert Committee on Biological Standardization
Fifty-ninth report.
WHO Technical Report Series, No. 964, 2012 (viii + 228 pages)

WHO Expert Committee on Biological Standardization
Fifty-eighth report.
WHO Technical Report Series, No. 963, 2011 (viii + 244 pages)

WHO Expert Committee on Biological Standardization
Fifty-seventh report.
WHO Technical Report Series, No. 962, 2011 (viii + 206 pages)

Website: http://www.who.int/biologicals
This report presents the recommendations of a WHO Expert Committee commissioned to coordinate activities leading to the adoption of international recommendations for the production and control of vaccines and other biological substances, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of general issues brought to the attention of the Committee. The next part of the report, of particular relevance to manufacturers and national regulatory authorities, outlines the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, a WHO guidance document on *Regulatory assessment of approved rDNA-derived biotherapeutics* was adopted along with WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions and on WHO good manufacturing practices for biological products. In addition, revised WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines were also adopted by the Committee.

Subsequent sections of the report provide information on the current status and proposed development of international reference materials in the areas of antibiotics; biotherapeutics other than blood products; blood products and related substances; in vitro diagnostic device reagents; and vaccines and related substances.

A series of annexes are then presented which include an updated list of all WHO Recommendations, Guidelines and other documents on biological substances used in medicine (Annex 1). The above four WHO documents adopted on the advice of the Committee are then published as part of this report (Annexes 2–5). Finally, all additions and discontinuations made during the 2015 meeting to the list of International Standards, Reference Reagents and Reference Panels for biological substances maintained by WHO are summarized in Annex 6. The updated full catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/.